BOX INTERFERENCE

Docket No.: DITI-109

T. TUCK #46 1/21/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS AND INTERFERENCES

O I P E
DEC 1 3 1999
JC55
PATENT & TRADEMARK OFFICE

Applicant : William McBride et al.
Serial No. : 08/253,973
Filed : June 3, 1994
Title : MONOAMINE, DIAMIDE, THIOL-CONTAINING METAL CHELATING AGENTS

Art Unit : 1211
Examiner : M. Hartley

RECEIVED
DEC 22 1999
TECH CENTER 1600/2900

BOARD OF PATENT APPEALS AND INTERFERENCES
1999 DEC 20 PM 12: 30
RECEIVED

BOX: Interference
Assistant Commissioner for Patents
Washington, D.C. 20231

REQUEST UNDER 37 C.F.R. § 1.607 FOR INTERFERENCE

Applicants respectfully request that an interference be declared between the above-identified application and U.S. Patents Nos. 5,662,885 and 5,780,006.

The instant application is on *ex parte* appeal to the Board of Patent Appeals and Interferences. A Reply Brief was telefaxed to the Patent and Trademark Office on 7 December 1999. **It is further requested that, pursuant to 37 C.F.R. § 1.607(b), proceedings in this appeal be conducted with special dispatch.**

Compliance with 37 C.F.R. § 1.607(a)

The information required by 37 C.F.R. § 1.607(a) is set forth under headings which correspond to the subsections of this Rule.

1. Identification of the Interfering Patents: The patents which claim subject matter interfering with the subject matter claimed in the instant application are U.S. Patents Nos. 5,662,885 ("the '885 patent") and 5,780,006 ("the '006 patent"), both titled "Peptide Derived Radionuclide Chelators" and issued to Alfred Pollak and Anne

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

December 8, 1999
Date of Deposit

Cynthia M. Berens
Signature

Cynthia M. Berens
Typed or Printed Name of Person Signing Certificate

Goodbody. Resolution Pharmaceuticals Inc. of Mississauga, Ontario, is named as the Assignee on the face of both patents. The '006 patent is a continuation of the '885 patent and the '006 patent has a terminal disclaimer so that (assuming all maintenance fees are paid) both patents will expire on the same day. Copies of the '885 and '006 patents are here attached as Appendices A and B, respectively.

2. Proposed Count: Applicants propose a single count reading:

Claim 1 of U.S. Patent No. 5,780,006

OR

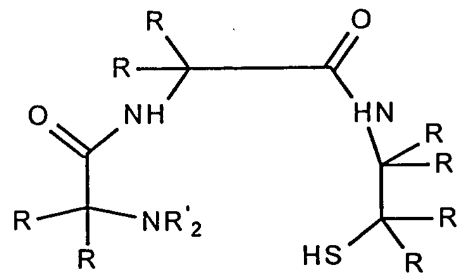
Claim 2 of U.S. Patent Application No. 08/253,973

Because of the different terminology in application No. 08/253,973 ("the '973 application") and in the '885 and '006 patents, it is believed that the most efficient way to set up this interference would be to use alternative claims as the Count. This procedure has become quite common in interferences involving biotechnology and has been explicitly endorsed in Hsing v. Myers, 2 U.S.P.Q. 2d 1861, fn 1 (BPAI 1986). The use of alternative claims as the Count ensures compliance with the requirement of 37 C.F.R. § 1.606 that, when an interference is initially declared, the Count should not be narrower in scope than any patentable application claim or any patent claim designated to correspond to the Count.

The following analysis is being furnished in order to show that the parties to the proposed interference are, in fact, claiming the same patentable invention.

In claim 2 of the '973 application, the digits represented by n, m and p can be each 0 or 1. If all three are 0, said claim would read:

2. A reagent comprising a targeting moiety covalently linked to a metal chelator having a formula:



wherein:

each R′ is independently H, lower alkyl, hydroxyalkyl (C_2-C_4), or alkoxyalkyl (C_2-C_4);

each R is independently H or R", where R" is substituted or unsubstituted lower alkyl or phenyl not comprising a thiol group;

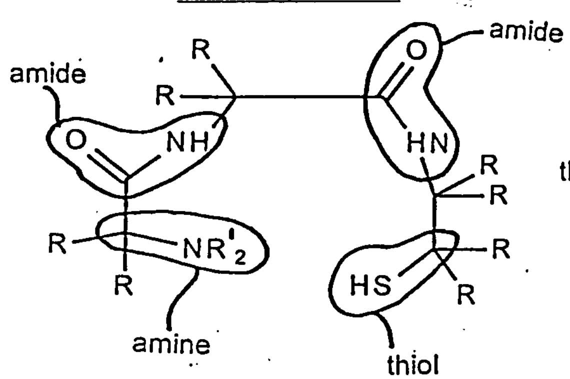
one R or R′ is L, wherein when an R′ is L, -NR′$_2$ is an amine; and

L is a bivalent linking group linking the chelator to the targeting moiety.

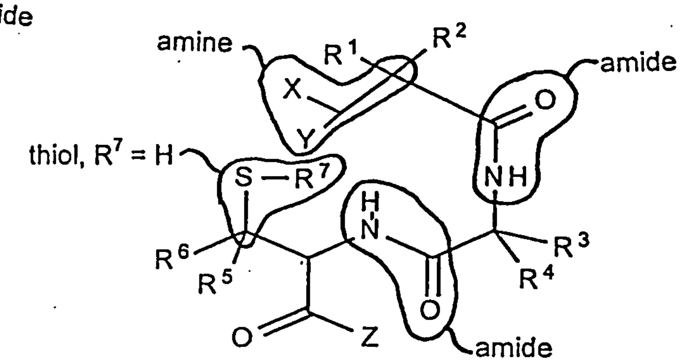
The essential feature of the chelating moiety in said claim is the presence of a single amine group, two amide groups and a single thiol group. This feature is also

present in claim 1 of the '006 patent as can be seen from the following side-by-side comparison of the two structural formulae:

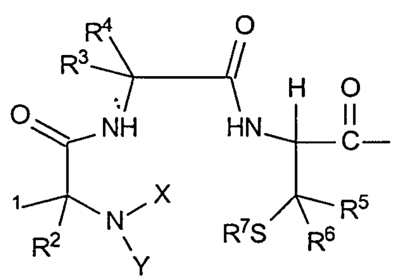
'973 Application



'885 and '006 Patents

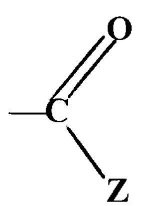


Now, if we use the terminology of the '885 and '006 patents and put them into the modified structural formula of the '973 application, we obtain a structure which appears as:



The following table shows the definitions of the various substituent groups in the '885 and '006 patents and in the '973 application which would be **common** to both parties:

'006 Patent Claim 1	'973 Application Claim 2
X is a linear . . . saturated . . . C_{1-4} alkyl chain that is optionally interrupted by . . . O, . . . and is optionally substituted by . . . hydroxyl . . .	**R′ is . . . lower alkyl, hydroxyalkyl (C_2-C_4) or alkoxyalkyl (C_2-C_4)**

'006 Patent Claim 1	'973 Application Claim 2
Y is H or a substituent defined by X	**R′ is . . . H, lower alkyl, hydroxyalkyl (C_2-C_4) or alkoxyalkyl (C_2-C_4)**
R′ through R^4 are selected independently from H; . . . C_{1-4} alkyl; C_{1-4} alkyl substituted with . . . hydroxyl; . . . and C(O)Z [Z = H]	**R is independently H or R" where R" is substituted or unsubstituted lower alkyl . . . not comprising a thiol group** ["substituted" includes hydroxyl (page 11, line 27) and R" includes carboxylic acid (page 11, line 29)]
R_5 and R_6 are selected independently from H; . . . C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl; . . . and C(O)Z [Z = H]	
R^7 is . . . H . . .	**H**
 [Z = H]	**R is** carboxylic acid (page 11, line 29)
Z is . . . a targeting molecule	**one of R . . . is L . . . and L is a bivalent linking group linking the chelator to the targeting moiety**

3. Claims in the Patents which Correspond to the Proposed Count: Claim 1 of the '006 patent corresponds exactly to the proposed Count. The remaining claims of the '006 patent and all of the claims in the '885 patent correspond substantially to the proposed Count.

4. Identification of Application Claims which Correspond to the Count and Explanation of the Application and Patent Claims which do not Correspond Exactly to the Count: Claim 2 of the '973 application corresponds exactly to the Count.

As noted above, all of the claims of the '885 patent and claims 2 through 22 of the '006 patent correspond substantially to the Count.

Claim 1 of the '885 patent does not permit the substituent Y to be a hydrogen atom and includes the additional limitation that the targeting molecule be a peptide. Claim 2 requires that the peptide comprise three or more amino acids. These claims correspond to the Count because the essential feature of the chelator – the amine/diamide/thiol chelating moiety remains the same.

Claim 3 of the '885 patent defines a compound in which the chelator of claim 2 of the '006 patent is complexed with a metal radionuclide or an oxide or nitride thereof. Claim 4 is a further limitation that the radionuclide be technetium or an oxide thereof. Complexing a chelator, including a chelator linked to a targeting moiety, such as a peptide, with a radionuclide is well known in the art. Claim 5 is analogous to claim 3 except that Y is not permitted to be hydrogen.

Claim 6 of the '885 patent inclues the combined limitations of claims 1 and 3. Claims 7-11 are ultimately dependent from claim 6. The essential features of the chelator remain the same and, therefore, these claims also correspond to the proposed Count.

Claim 12 of the '885 patent claims a specific chelator complexed to a metal radionuclide. Claim 13 limits the radionuclide to technetium or an oxide thereof. The chelating portion of the compound:

N,N-dimethylGly—Thr—Cys(acm) . . .

conforms to the requirements of claim 1 of the '006 patent.

Claims 14 and 15 of the '885 patent are essentially the same as claims 6 and 8, respectively, except that Y is not permitted to be a hydrogen atom.

Claims 16 through 18 of the '885 patent are directed to the compound in which the chelating moiety is

N,N-dimethylGly—Ser—Cys(acm) . . .

which falls within the structural limitations of claim 2 of the '006 patent.

In the '006 patent, claims 2 through 8 define various substituents of the structural formula in claim 1 and thus are directed to specific amino acid moieties. The essential amine/diamide/thiol chelate structure is not affected and therefore these claims correspond to the proposed Count.

Claim 9 of the '006 patent limits Z to a "targeting molecule" and, based on disclosure of this patent, it appears that it is intended that only one Z be a targeting molecule. Claim 10 requires that the targeting molecule be a peptide. Claim 11 requires that the peptide comprise three or more amino acid residues. Claims 12 and 13 are directed to specific peptide sequences. For reasons discussed above as to certain dependent claims of the '885 patent, these claims of the '006 patent also correspond to the proposed Count.

Claim 14 of the '006 patent is directed to a specific compound. The chelating moiety

N,N-dimethylGly—Thr—Cys(acm) . . .

is included in the structure of claim 2.

Claims 15 through 22 of the '006 patent are method claims directed to the use of compounds falling within the scope of claim 1. These methods are not patentably distinct from the compounds themselves.

As noted above, claim 2 of the '973 application would correspond exactly to the Count. All of the remaining claims in the '973 application are dependent from claim 2. All claims in the application have been rejected under 35 U.S.C. § 103(a). The question of which of these dependent claims would correspond to the proposed Count can be determined only after outcome of the appeal proceedings.

5. Application of the Terms of Newly-Presented Claims in the Application to the Disclosure: No claims are being added.

6. Requirements of 35 U.S.C. § 135(b): The '885 patent was issued on 2 September 1997. Claim 2 of the '973 application was an original claim, and said application was filed on 3 June 1994. Amendments have been made in claim 2, but none of them enlarge the scope of the claim.

Priority

In the proposed interference, Applicants would be entitled to the benefit of application No. 08/092,355 filed on 15 July 1993 and of application No. 08/095,760 filed on 21 July 1993. A copy of application No. 08/092,355 as filed is here attached as Appendix C. Pages 19 and 20, which were difficult to read as originally filed, are replaced by new pages 19a, 19b, 20a and 20b. These new pages were submitted with an amendment filed on 27 October 1998. A copy of U.S. Patent No. 5,620,675, which issued on application No. 08/095,760, is attached as Appendix D.

On 24 June 1999, Applicants amended the instant application to claim priority from said two earlier-filed applications.

The priority claim is based on the fact that both earlier applications disclose reagents within the scope of Applicants' claims. The following table identifies the reagents in the '973 application application and shows where they also appear in application No. 08/092,355 and in U.S. Patent No. 5,620,675.

'973 Application	Application No. 08/092,355	U.S. Patent No. 5,620,675
page 23, line 13		col. 11, line 52
page 23, line 14	page 19a, 2nd line	
page 23, line 15	page 18, line 26	
page 23, line 25		col. 11, line 44
page 23, line 26		col. 11, line 55
page 23, line 27		col. 11, line 45
page 23, line 28		col. 11, line 40
page 23, line 29		col. 11, line 48
page 23, line 31		col. 11, line 31
page 23, line 32		col. 11, line 37
page 23, line 33		col. 11, line 39
page 23, line 34	page 18, line 21	
page 23, line 35		col. 11, line 35

It is therefore requested that in the proposed interference, Applicants be accorded the benefit of these earlier filed application.

Status of the Parties in the Proposed Interference

The effective filing date for both the '885 and the '006 patent is 22 July 1994. The '973 application was filed on 3 June 1994 and, as discussed above, is a continuation-in-

part of two earlier applications. Therefore, in the proposed interference, Applicants will be senior party.

Respectfully submitted,

Date: 8 December 1999

Frederick H. Rabin

Frederick H. Rabin
Reg. No. 24,488

Fish & Richardson P.C.
45 Rockefeller Plaza, Suite 2800
New York, NY 10111
Telephone: (212) 765-5070
Facsimile: (212) 258-2291

30003960.doc

A

US005662885A

United States Patent [19]

Pollak et al.

[11] Patent Number: 5,662,885

[45] Date of Patent: Sep. 2, 1997

[54] **PEPTIDE DERIVED RADIONUCLIDE CHELATORS**

[75] Inventors: **Alfred Pollak; Anne Goodbody**, both of Toronto, Canada

[73] Assignee: **Resolution Pharmaceuticals Inc.**, Ontario, Canada

[21] Appl. No.: **279,155**

[22] Filed: **Jul. 22, 1994**

[51] **Int. Cl.^{6}** **A61K 51/00**; A61M 36/14

[52] **U.S. Cl.** **424/1.69**; 534/14; 530/300; 530/328; 530/329; 530/330

[58] **Field of Search** 424/1.69, 1.11, 424/1.65; 530/328, 300, 324–330; 534/10–14, 7; 540/1, 450; 544/1, 63, 224; 546/1, 152, 184, 249, 250; 548/100, 215, 300.1, 400

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,390,528	6/1983	Najjar	424/177
4,720,484	1/1988	Vincent	514/18
4,957,736	9/1990	Nencioni	424/88
4,965,250	10/1990	Vincent	514/18
4,965,392	10/1990	Fritzberg	558/254
4,980,147	12/1990	Fritzberg	424/1.1
4,986,979	1/1991	Morgan, Jr. et al.	424/1.11
5,021,567	6/1991	Johnson et al.	540/470
5,028,593	7/1991	Nishioka	514/18
5,043,423	8/1991	Viscomi	530/344
5,047,400	9/1991	Vincent	514/88
5,082,930	1/1992	Nicolotti	530/402
5,091,514	2/1992	Fritzberg	534/14
5,112,594	5/1992	Woulfe	424/1.1
5,175,257	12/1992	Kasina	530/391.5
5,187,264	2/1993	Verbruggen	534/14
5,202,109	4/1993	Fritzberg	424/1.1
5,202,451	4/1993	Fritzberg	556/419
5,248,764	9/1993	Flanagan	530/324
5,480,970	1/1996	Pollak et al.	530/328
5,569,745	10/1996	Goodbody et al.	530/328

FOREIGN PATENT DOCUMENTS

454302	12/1991	European Pat. Off.	C07K 5/02
569132	11/1993	European Pat. Off.	C07F 13/00
WO 90/09172	8/1990	WIPO .	
WO 9213572	8/1992	WIPO	A61K 49/02
WO 9219274	11/1992	WIPO	A61K 49/02
9503280	2/1995	WIPO .	

OTHER PUBLICATIONS

Dewanjee et al. Seminars in Nuclear Medicine vol. XX, No. 1 (Jan.), 1990:5–27 "The chemistry of ^{99m}Tc-labeled radiopharmaceuticals".

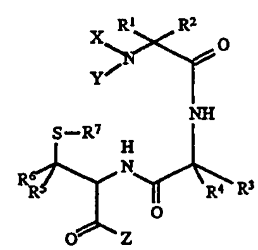
Fridkin et al Mol Cell Biochem, 1981, 41:73 "Tuftsin, Thr-Lys-Pro-Arg".

Miller J nucl Med, 1993, 34[11]:15N "Synthetic peptides come of age".

Primary Examiner—John Kight
Assistant Examiner—Dameron L. Jones
Attorney, Agent, or Firm—Nikaido Marmelstein Murray & Oram LLP

[57] **ABSTRACT**

For use in imaging sites of diagnostic interest within the body, the present invention provides radionuclide chelators, optionally coupled to targeting molecules such as peptides of the formula:



18 Claims, 1 Drawing Sheet

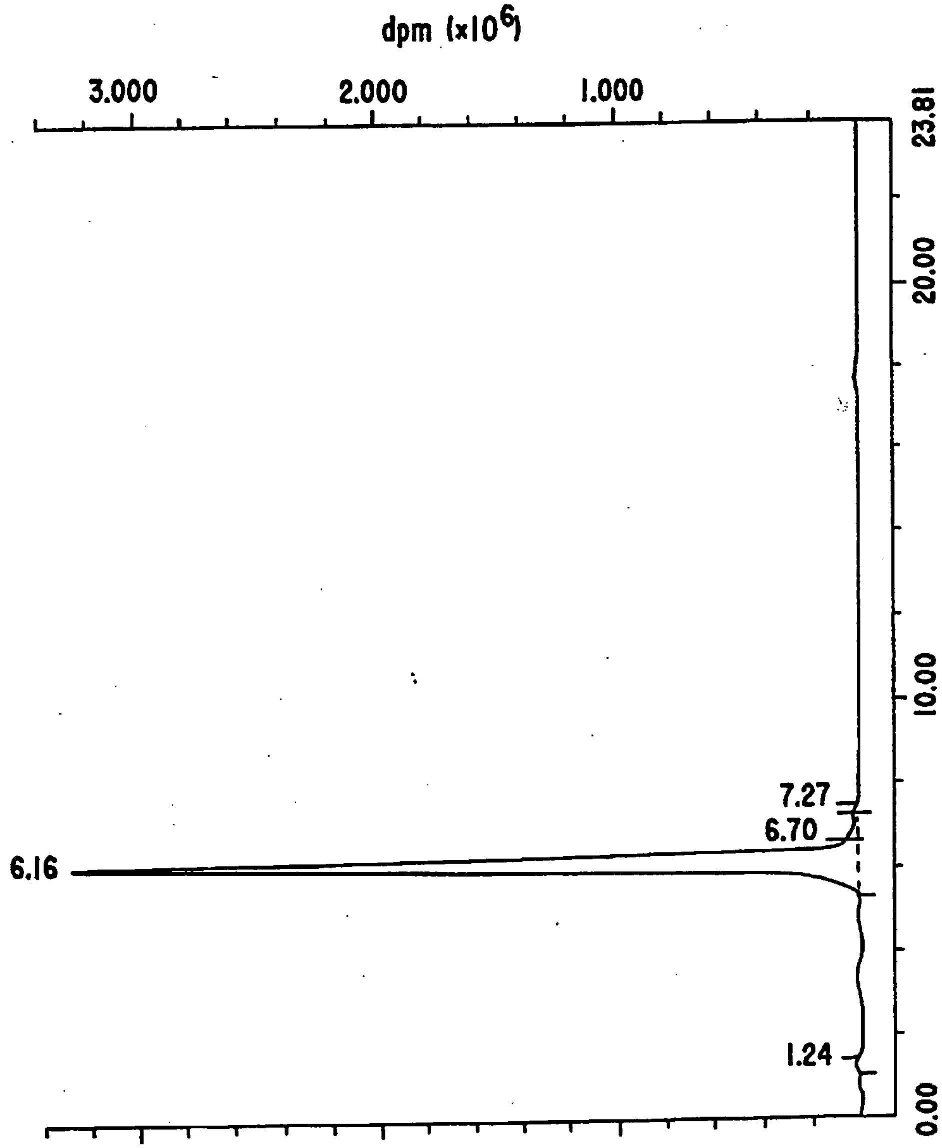


Fig. I

PEPTIDE DERIVED RADIONUCLIDE CHELATORS

FIELD OF THE INVENTION

This invention is in the field of diagnostic imaging, and relates to chemical chelators useful in the radiolabeling of agents that target tissues of diagnostic interest.

BACKGROUND OF THE INVENTION

The art of diagnostic imaging exploits contrasting agents that in binding or localizing site selectively within the body, help to resolve the image of diagnostic interest. ^{67}Gallium salts, for example, have an affinity for tumours and infected tissue and, with the aid of scanning tomography, can reveal afflicted body regions to the physician. Other contrasting agents include the metal radionuclides such as ^{99m}technetium and $^{186/188}$rhenium, and these have been used to label targeting molecules, such as proteins, peptides and antibodies that localize at desired regions of the human body.

As targeting agents, proteins and other macromolecules can offer the tissue specificity required for diagnostic accuracy; yet labeling of these agents with metal radionuclides is made difficult by their physical structure. Particularly, protein and peptide targeting agents present numerous sites at which radionuclide binding can occur, resulting in a product that is labeled heterogeneously. Also, and despite their possibly large size, proteins rarely present the structural configuration most appropriate for high affinity radionuclide binding, i.e. a region incorporating four or more donor atoms that form five-membered rings. As a result, radionuclides are bound typically at the more abundant low-affinity sites, forming unstable complexes.

To deal with the problem of low affinity binding, Paik et al (Nucl Med Biol 1985, 12:3) proposed a method whereby labeling of antibodies is performed in the presence of excess DPTA (diaminetrimethylenepentaacetic acid), to mask the low affinity binding sites. While the problem of low affinity binding is alleviated by this method, actual binding of the radionuclide, in this case technetium, was consequently also very low. The direct labeling of proteins having a high proportion of cysteine residues also has been demonstrated (Dean et al; WO 92/13,572). This approach exploits thiol groups of cysteine residues as high-affinity sites for radionuclide binding, and is necessarily limited in application to those targeting agents having the required thiol structure.

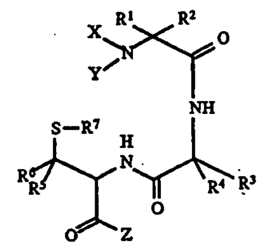
A promising alternative to the direct labeling of targeting agents is an indirect approach, in which targeting agent and radionuclide are coupled using a chelating agent. Candidates for use as chelators are those compounds that bind tightly to the chosen metal radionuclide and also have a reactive functional group for conjugation with the targeting molecule. For use in labeling peptide and protein-based targeting agents, the chelator is ideally also peptide-based, so that the chelator-targeting molecule conjugate can be synthesized in toto using peptide synthesis techniques. For utility in diagnostic imaging, the chelator desirably has characteristics appropriate for its in vivo use, such as blood and renal clearance and extravascular diffusibility.

SUMMARY OF THE INVENTION

The present invention provides chelators that bind diagnostically useful metal radionuclides, and can be coupled to targeting agents capable of localizing at body sites of diagnostic and therapeutic interest. The chelators of the present invention are peptide analogs designed structurally

to present an N_3S configuration capable of binding oxo, dioxo and nitrido ions of ^{99m}technetium and $^{186/188}$rhenium.

More particularly, and according to one aspect of the invention, there are provided metal radionuclide chelators of the formula:



(I)

wherein

- X is a linear or branched, saturated or unsaturated C_{1-4}alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4}alkyl, aryl and C(O)Z;
- Y is H or a substituent defined by X;
- X and Y may together form a 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, oxo, C_{1-4}alkyl, aryl and C(O)Z;
- R^1 through R^4 are selected independently from H; carboxyl; C_{1-4}alkyl; C_{1-4}alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4}alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;
- R^5 and R^6 are selected independently from H; carboxyl; C_{1-4}alkyl; C_{1-4}alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;
- R^7 is selected from H and a sulfur protecting group; and
- Z is selected from hydroxyl and a targeting molecule.

According to another aspect of the invention, the chelators of the above formula are provided in a form having the metal radionuclide complexed therewith.

In another aspect of the invention, there is provided a conjugate in which the chelator is provided in a form coupled to a diagnostically useful targeting molecule, and optionally in combination with a complexed metal radionuclide, for imaging use.

In another aspect of the invention, there is provided a method of imaging sites of diagnostic interest in which a conjugate of the invention is first administered as a radionuclide complex to a patient; and then the location of the radionuclide is detected using imaging means.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an HPLC analysis of conjugate N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg-OH [SEQ ID NO: 1] labeled with ^{99m}Tc.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides metal radionuclide chelators that when coupled to a targeting molecule are useful for delivering a radionuclide to a body site of therapeutic or diagnostic interest. As illustrated in the above formula, the chelators are peptidic compounds that present an N_3S configuration in which the radionuclide is complexed.

Terms defining the variables R^1–R^7, X, Y and Z as used hereinabove have the following meanings:

"alkyl" refers to a straight or branched C_{1-4} chain;

"aryl" refers to aromatic and heteroaromatic rings;

"halogen" refers to F, Cl and Br;

"sulfur protecting group" refers to a chemical group that inhibits oxidation of a thiol group, which includes those that are cleaved upon chelation of the metal. Suitable sulfur protecting groups include known alkyl, aryl, acyl, alkanoyl, aryloyl, mercaptoacyl and organothio groups.

In preferred embodiments of the invention, the chelators conform to the above formula in which:

R^1 through R^4 are selected independently from H; and a hydroxy-substituted C_{1-4}alkyl group such as hydroxymethyl and 1-hydroxyethyl;

R^5 and R^6 are selected independently from H and C_{1-4}alkyl, and are preferably both H;

R^7 is a hydrogen atom or a sulfur protecting group and is most preferably acetamidomethyl;

X is a C_{1-4}alkyl chain, preferably methyl or ethyl; or is a C_{1-4}alkyl chain substituted with an aryl group, preferably benzyl;

Y is H or a substituent defined by X; and is preferably methyl or ethyl and most preferably the same as X;

Z is OH or a targeting molecule, and is preferably a peptide targeting molecule.

Specific chelators of the invention include:

N,N-dimethylGly-Ser-Cys(Acm)-Z; and

N,N-dimethylGly-Thr-Cys(Acm)-Z-OH.

In the case where the substituents represented by X and Y together with the adjacent nitrogen atom form a hetero ring, such a ring may be a 5- to 8-membered, saturated ring, for example pyrrolidine, piperdine, 1-azacycloheptane and 1-azacyclooctane. Unsaturated rings formed by X and Y include pyrrole and 4H-pyridine while it is understood that the coordinating nitrogen of the ring is necessarily trivalent and cannot form a double bond to an adjacent atom. The heterocycle formed by X and Y may also incorporate one or two additional heteroatoms selected from N, O and S. Rings having additional hetaroatoms include but are not limited 1-imidazole, pyrazole, piperazine, morpholine and thiomorpholine. The ring formed by X and Y may also be substituted with one or more and preferably less than three groups selected from halogen, hydroxyl, carboxyl, oxo, C_{1-4}alkyl and aryl, for example to form 4-oxo-1-piperidine, 4-oxo-1-pyrrolidine and 4-hydroxy-1-piperidine.

For diagnostic imaging purposes, the chelator pre se may be used in a form complexed with a metal radionuclide. Suitable radionuclides include technetium and rhenium in their various forms such as ReO^{3+}, ReO_2^+, $^{99m}TcO_2^+$ and most preferably $^{99m}TcO^{3+}$. Desirably and according to a preferred aspect of the invention, the chelator is coupled to a targeting molecule represented by Z in the above formula, to form a conjugate that serves to deliver a chelated radionuclide to a desired location in a mammal. Examples of targeting molecules suitable for coupling to the chelator include, but are not limited to, steroids, proteins, peptides, antibodies, nucleotides and saccharides. Preferred targeting molecules include proteins and peptides, particularly those capable of binding with specificity to cell surface receptors characteristic of a particular pathology. For instance, disease states associated with over-expression of particular protein receptors can be imaged by labeling that protein or a receptor binding fragment thereof coupled to a chelator of invention. Most preferably targeting molecules are peptides capable of specifically binding to target sites and have three or more amino acid residues. Targeting peptides useful to image certain medical conditions and tissues are noted below.

for atherosclerotic plaque:	
YRALVDTLK [SEQ ID NO: 2]	RALVDTLK [SEQ ID NO: 3]
RALVDTLKFVTQAEGAK [SEQ ID NO: 4]	YAKFRETLEDTRDRMY [SEQ ID NO: 5]
AKFRETLEDTRDRMY [SEQ ID NO: 6]	AALDLNAVANKIADFEL [SEQ ID NO: 7]
YAALDLNAVANKIADFEL [SEQ ID NO: 8]	YRALVDTLKFVTEQAKGA [SEQ ID NO: 9]
RALVDTLKFVTEQAKGA [SEQ ID NO: 10]	YRALVDTEFKVKQEAGAK [SEQ ID NO: 11]
RALVDTEFKVKQEAGAK [SEQ ID NO: 12]	YRALVDTLKFVTQAEGAK [SEQ ID NO: 13]
for Infections and atherosclerotic plaque:	
VGVAPGVGVAPGVGVAPG [SEQ ID NO: 14]	formyl.Nleu.LF.Nleu.YK [SEQ ID NO: 15]
VPGVGVPGVGVPGVGVPGVG [SEQ ID NO: 16]	formylMIFL [SEQ ID NO: 17]
formylMLFK [SEQ ID NO: 18]	formylMLFI [SEQ ID NO: 19]
formylMFIL [SEQ ID NO: 20]	formylMFLI [SEQ ID NO: 21]
formylMLIF [SEQ ID NO: 22]	formylMILF [SEQ ID NO: 23]
formylTKPR [SEQ ID NO: 24]	VGVAPG [SEQ ID NO: 25]
formylMLF [SEQ ID NO: 26]	YIGSR
CH_2CO.YIGSRC [SEQ ID NO: 27]	
for thrombus:	
NDGDFEEIPEEYLQ [SEQ ID NO: 28]	NDGDFEEIPEEY(SO_3Na)LQ [SEQ ID NO: 29]
GPRG [SEQ ID NO: 30]	
for platelets:	
D-Phe.PRPGGGGNGDFEEIPEEYL [SEQ ID NO: 31]	RRRRRRRRRGDV [SEQ ID NO: 32]
PLYKKIIKKLLES [SEQ ID NO: 33]	RGD
RGDS [SEQ ID NO: 34]	
for amyloid plaque (Alzheimer's disease):	
EKPLQNFTLSFR [SEQ ID NO: 35]	

For connection to the chelator, a targeting molecule may comprise a "spacer" that serves to create a physical separation between the chelator and the targeting molecule. A spacer may be an alkyl chain that is derivatized for coupling

to the chelator. In the case where the targeting molecule is a peptide, the spacer may suitably be one or more amino acid residues. Preferably, peptidic targeting molecules incorporate spacers of from 1 to 5 amino acids such having chemically inert α-carbon side chains, such as glycine or β-alanine residues.

A targeting molecule may be coupled to a chelator of the invention at various sites including R^1 to R^6, X, Y and Z as well as a ring formed by X and Y. Coupling may be achieved by reacting a group present on the targeting molecule that is reactive with a substituent on the chelator to form a linkage. For example, peptide targeting molecules having a free amino group, such as an N-terminus or an ε-amino-lysine group may be reacted with a carboxyl group on the chelator to form an amide linkage. Alternatively, the C-terminus of the peptide targeting molecule may be reacted with an amino substituent on the chelator. In a preferred embodiment, targeting molecules are coupled to chelators of formula (I) at substituent Z by an amide linkage such as a peptide bond. For example, the N-terminus amino group of a peptide targeting molecule is reacted with a carboxyl group at Z. Targeting molecules other than peptides may be coupled to chelators of the invention in a similar manner provided that a group suitable for coupling to the chelator is present. In the instance that a suitable group is not present, the targeting molecule may be chemically derivatized to present such a group. When more than one reactive group is present on the chelator or targeting molecule. It is desirable to block all but the particular group for coupling with an appropriate blocking agent in order to achieve a single conjugate species. For example, free carboxyl groups may be protected by forming esters such as a t-butyl ester which can be removed with TFA. Free amino groups may be protected with a blocking group such as FMOC which may be subsequently removed with piperdine.

In a particular embodiment of the invention, imaging in vivo sites of focal inflammation is accomplished using a conjugate in which the targeting molecule is a chemotactic peptide comprising the amino acid sequence Thr-Lys-Pro-Pro- Arg(TKPPR) [SEQ ID NO:36]. It has been found that this peptide binds particularly well to leukocytes receptors. Targeting peptides can be spaced from the chelator by additional amino acid residues, preferably glycine, provided the peptide retains its localizing activity. In a particular embodiment, the peptide TKPPR [SEQ ID NO:36] is coupled to substituent Z of chelators according to formula (I) by a Gly residue.

Peptide-based targeting molecules can be made, either per se of as a conjugate with a chelator, using various established techniques. Because it is amenable to solid phase synthesis, employing alternating FMOC protection and deprotection is the preferred method of making short peptides. Recombinant DNA technology is preferred for producing proteins and long fragments thereof. In a particular embodiment, peptide-chelator conjugates are prepared by solid-phase peptide synthesis methods, which involve the stepwise addition of amino acid residues to a growing peptide chain that is linked to an insoluble (solid) support or matrix, such as polystyrene. The C-terminal residue of the targeting peptide is first anchored to a commercially available support with its amino group protected with an N-protecting agent such as a fluorenylmethoxycarbonyl (FMOC) group. Typically, the support is obtained with the C-terminal residue preloaded in protected form. The amino protecting group is removed with suitable deprotecting agents such as piperidine and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclocarbodiimide (DCC). Upon formation of a peptide bond, the reagents are washed from the support. Once the targeting peptide chain is synthesized, the first residue of the chelator ie. S-acetamidomethyl protected cysteine is added to the N-terminus. The final residue of the chelator is a derivatized amino acid residue that conforms to the formula (X)(Y)N—C(R^1)(R^2)—CO— wherein X, Y, R^1 and R^2 have the meaning previously defined. The final residue, for example dimethyl-glycine or sarcosine, may be commercially obtained or may be synthesized. The completed conjugate is cleaved from the support with a suitable reagent such as trifluoroacetic acid (TFA).

It will be appreciated that all substituents R^1 through R^4 according to the invention are side chains of naturally occurring or derivatized amino acids including D-amino acids and are commercially available and compatible with solid phase synthesis techniques. Derivatized amino acid residues that are not commercially available may be incorporated in chelators of the invention by synthesizing them according to established organic chemistry techniques and inserting at the appropriate stage of solid phase peptide synthesis previously described. Similarly when substituents R^5 and R^6 are other than H, a derivatized cysteine amino acid residue is utilized in the peptide synthesis. For example, the commercially available residue penecillamine is incorporated when R^5 and R^6 are both methyl.

Various substituents at X and Y may be introduced in chelators of the invention by incorporating as the final residue of solid phase synthesis a derivatized amino acid according to the formula (X)(Y)N—C(R^1)(R^2)—C(O)—OH wherein X, Y, R^1 and R^2 have the meaning previously described. Amino acids having N-terminal amino substituents according to X and Y may be synthesized according to established organic chemistry procedures and techniques. For example, when X and Y are both dibenzyl substituents the corresponding dibenzylglycine residue may be synthesized by reacting commercially available reagents bromoacetic acid and dibenzylamine in a suitable solvent such as dichloromethane and then heating. Other amines may be employed in the reaction in place of dibenzylamine such as diisopropylamine to give the corresponding diisopropylglycine. Similarly cyclic amines such as piperidine and morpholine in place of dibenzylamine will give the corresponding piperidylglycine and morpholinylglycine residues.

In a most preferred embodiment of the invention a peptide-chelator conjugate is prepared on a solid support and has the structure of formula (I) wherein the targeting molecule is a peptide having a sequence Gly-Thr-Lys-Pro-Pro-Arg-OH [SEQ ID NO:37]; R^1, R^2, R^3, R^5 and R^6 are H; R^4 is hydroxymethyl or 1-hydroxyethyl and R^7 is acetamidomethyl. Incorporation of the selected radionuclide within the chelator can be achieved by various established methods. For example the following general procedure may be used. A chelator solution is formed initially by dissolving the chelator in aqueous alcohol eg. ethanol-water 1:1. Oxygen is removed for example by degassing with N_2, then sodium hydroxide is added to remove the thiol protecting group. The solution again purged of oxygen and heated on a water bath to hydrolyze the thiol protecting group, and the solution is then neutralized with an organic acid such as acetic acid (pH 6.0–6.5). In the labeling step, sodium pertechnetate is added to a chelator solution with an amount of stannous chloride sufficient to reduce the technetium. The solution is mixed and left to react at room temperature and then heated on a water bath. In an alternative method, labeling can be accomplished with the chelator solution adjusted to pH 8. At this higher pH, pertechnetate may be replaced with a solution

containing technetium complexed with labile ligands suitable for ligand exchange reactions with the desired chelator. Suitable ligands include tartarate, citrate and heptagluconate. Stannous chloride may be replaced with sodium dithionite as the reducing agent if the chelating solution is alternatively adjusted to a still higher pH of 12–13 for the labeling step. The chelators of the present invention can be coupled to a targeting molecule prior to labeling with the radionuclide, a process referred to as the "bifunctional chelate" method. An alternative approach is the "prelabeled ligand" method in which the chelator is first labeled with a radionuclide and is then coupled to the targeting molecule.

The labeled chelator may be separated from contaminants $^{99m}TcO_4^-$ and colloidal $^{99m}TcO_2$ chromatographically, e.g., with a C-18 Sep Pak column activated with ethanol followed by dilute HCl. Eluting with dilute HCl separates the $^{99m}TcO_4^-$, and eluting with EtOH-saline 1:1 brings off the chelator while colloidal $^{99m}TcO_2$ remains on the column.

When coupled to a targeting molecule and labeled with a diagnostically useful metal, chelators of the present invention can be used to detect pathological conditions by techniques common in the art of diagnostic imaging. A chelator-targeting molecule conjugate labeled with a radionuclide metal such as technetium may be administered to a mammal intralymphatically, intraperitoneally and preferably intravenously in a pharmaceutically acceptable solution such as saline or blood plasma medium. The amount of labeled conjugate administered is dependent upon the toxicity profile of the chosen targeting molecule as well as the profile of the metal and is typically in the range of about 0.01 to 100 and preferably 10 to 50 mCi per 70 Kg host. Localization of the metal in vivo is tracked by standard scinitigraphic techniques at an appropriate time subsequent to its administration. The time at which an image may be obtained will depend upon the profile of the targeting molecule, for example most peptides will localize rapidly allowing for an image to be obtained within 3 hours and often within 1 hour. In a particular embodiment, chelators of the invention coupled to a peptide targeting molecule GTKPPR in a saline solution are administered by intravenous injection to image sites of focal inflammation.

The following examples are presented to illustrate certain embodiments of the present invention.

EXAMPLE 1

Preparation of Peptide-Chelator Conjugates N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:1] and N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:38]

The title conjugates were prepared as a single peptide chain by solid phase peptide synthesis using FMOC chemistry on an FMOC-arginine preloaded 2-methoxy-4-alkoxybenzyl alcohol resin (Sasrin Resin, Bachem Biosciences In., Philadelphia) with an Applied Biosystems 433A peptide synthesizer (Foster City, Calif.). Derivatized amino acid residues S-acetamldomethyl-cysteine (Bachem) and N,N-dimethylglycine (Sigma Chemical Company, St. Louis, Mo.) were incorporated at the appropriate step of chain. elongation.

Upon addition of the final residue N,N-dimethylGly, the peptide-resin was dried under vacuum overnight and cleavage of the peptide from the resin was achieved by mixing a cooled solution of 9.5 mL trifluoroacetic acid (TFA), 0.5 mL water, 0.5 mL thioanisole and 0.25 mL 2-ethanedithiol (1 mL per 100 mg of peptide-resin) with the peptide-resin for 1.5 to 2 hours at room temperature. The resin was removed by filtration and washed with 1–3 mL of TFA to obtain 6–8 mL of a clear yellow liquid. This liquid was slowly dropped into 30–35 mL of cold tert-butyl ether in a 50 mL conical polypropylene centrifuge tube forming a white precipitate. The precipitate was centrifuged at 7000 rpm, 0° C. for 5 minutes (Sorvall RT6000, Dupont), decanted and washed two more times with tert-butyl ether. Following drying under vacuum the precipitate was dissolved in water. The solution was frozen in acetone-dry ice and lyophilized overnight. The resulting white powder was dissolved in water, filtered through a 0.45 μm syringe filter (Gelman Acrodisc 3 CR PTFE), and purified by reversed-phase HPLC (Beckman System Gold) with a C18 column (Waters RCM 25×10) using 1% TFA in water as buffer A and 1% TFA in acetonitrile as buffer B. The column was equilibrated with 100:0 buffer A:buffer B and eluted with a linear gradient in 25 minutes at 1 mL/min to 50% buffer B. Fractions were reanalysed on the HPLC and pooled according to matching profiles. The pure fractions were frozen in acetone-dry ice and lyophilized 12 hours to give a white powder.

EXAMPLE 2

Labeling of Peptide-Chelator Conjugates N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:1] and N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:38]

The conjugates of example 1 were reconstituted (200 μL; 1 mg/mL saline) and then injected into 3 mL vacutainers with 100 μL pertechnetate (10 mCi) and 100 μL stannous gluconate (50 μg stannous chloride and 1 mg sodium gluconate). The tubes were placed in boiling water bath for 12 minutes and then filtered through a Whatman PVDF syringe filter to collect the labeled conjugate solutions which were further diluted with saline to prepare injectable solutions (2 Mbq/mL). The conjugates were isolated by HPLC (Beckman) from a (20 μL) sample (before dilution) to determine the labeling yield by measuring radioactivity. Each conjugate gave a single peak and greater than 94% labeling yield. 24hours after labeling, N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:1] was reanalyzed on HPLC. No degradation or radiolysis products were observed.

EXAMPLE 3

In vivo imaging and Biodistribution of Conjugates

Rat inflammation studies were performed as follows. 2 male Wistar rats (Charles River, 200–250 g) were injected intramuscularly with (25 mg) zymosan, a yeast cell wall suspension, into their left hindlegs 24 hours before imaging. Focal inflammation in the leg was visually detectable after 1 day. 1 mg (ca. 0.7 μMol) of the chelator-peptide conjugate was dissolved in 50 μL of dimethylsulfoxide and added to an ethanol-water mixture (1:1, 200 μL). An aliquot of Tc-99m tartarate (ca. 400 MBq) was added and transchelation allowed to proceed for 20 min. at 100° C. The Tc-99m labeled conjugate was purified by solution through a Sep Pak cartridge and then diluted with saline to prepare an injectable formulation (200 μL) containing about 100 μCi (3.7 MBq) of activity.

The rats were anaesthetized with somnitol (40 to 50 mg/kg), and the labeled conjugate solution (200 μL) was injected intravenously via the tail vein. Serial whole-body scintigrams were acquired at 30 minutes. The rats were then killed with anaesthesia overdose and samples of organs,

urine, blood, inflamed muscle (left leg) and non-inflamed muscle (right leg) and inflammatory exudate (fluid) were weighed and counted in either a well-type gamma counter or in a gamma dose calibrator depending upon the organ. The dose calculations were made based on the assumption that the blood volume constituted 8% of body weight. The results of the conjugates represented in the table below are averages for two rats and are corrected for the residual dose in the tail.

Both conjugates gave excellent scinitigraphic images in comparison to other known inflammation imaging agents such as Ga-67, ^{99m}Tc-IgG, ^{111}In-WBC and ^{99m}Tc-Nanocoil which is indicated by the high target to background ratios (inflamed:uninflamed muscle) observed. The conjugates imaged much more rapidly than the known agents and exhibited superior biodistribution. Also, non-target organs such as liver and GI tract showed low accumulation.

Imaging Agent	Inflam:Uninfl	Fluid:Blood	Urine (% dose)	Liver (% dose)	GI Tract (% dose)
N,N-dimethylGly-Ser-[SEQ ID NO: 1] Cys(Acm)-GTKPPR-OH	5.3	1.9	63.5	2.4	2.8
N,N-dimethylGly-Thr-[SEQ ID NO: 38] Cys(Acm)-GTKPPR-OH	4.6	1.6	68.5	2.4	2.5
^{67}Ga	2.5	0.1	5.5	26.5	8.4
^{99m}Tc-IgG	2.8	0.03	1.2	17.6	0.7
^{111}In-WBC	1.5	0.1	0.2	36.9	3.6
^{99m}Tc-Nanocoll	3.3	0.2	0.8	66.7	2.1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 38

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Gly at position 1 has an N,N-dimethyl group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Cys at position 3 has an Acm group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Arg at position 10 is unsubstituted or has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gly Ser Cys Gly Thr Lys Pro Pro Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Arg Ala Leu Val Asp Thr Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Ala Leu Val Asp Thr Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Gln Ala Glu Gly Ala
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Ala Lys Phe Arg Glu Thr Leu Glu Asp Thr Arg Asp Arg Met Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Lys Phe Arg Glu Thr Leu Glu Asp Thr Arg Asp Arg Met Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Ala Leu Asp Leu Asn Ala Val Ala Asn Lys Ile Ala Asp Phe Glu
1 5 10 15

Leu

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Tyr Ala Ala Leu Asp Leu Asn Ala Val Ala Asn Lys Ile Ala Asp Phe
1 5 10 15

Glu Leu

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Glu Gln Ala Lys
1 5 10 15

Gly Ala

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Glu Gln Ala Lys Gly
1 5 10 15

Ala

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Tyr Arg Ala Leu Val Asp Thr Glu Phe Lys Val Lys Gln Glu Ala Gly
1 5 10 15

Ala Lys

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Arg Ala Leu Val Asp Thr Glu Phe Lys Val Lys Gln Glu Ala Gly Ala
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Tyr Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Gln Ala Glu Gly
1 5 10 15

Ala Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val Gly Val Ala Pro Gly Val Gly Val Ala Pro Gly Val Gly Val Ala
1 5 10 15

Pro Gly

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Leu at position 1 is formylNorLeu."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Leu at position 4 is NorLeu."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Leu Phe Leu Tyr Lys

1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
1               5                   10                  15

Pro Gly Val Gly
            20
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Ile Phe Leu
1
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has at formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Leu Phe Lys
1
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position has a

formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Leu Phe Ile
1

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Phe Ile Leu
1

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Phe Leu Ile
1

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Leu Ile Phe
1

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ile Leu Phe
1

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Tyr at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Lys Pro Arg
1

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Val Gly Val Ala Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Tyr Ile Gly Ser Arg
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Tyr at position 1 has a CH2CO group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Tyr Ile Gly Ser Arg Cys
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Asn Asp Gly Asp Phe Glu Glu Ile Pro Glu Glu Tyr Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Asn Asp Gly Asp Phe Glu Glu Ile Pro Glu Glu Tyr Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Pro Arg Gly
1

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Pro at position 1 has a D-Phe group."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Pro Arg Pro Gly Gly Gly Gly Asn Gly Asp Phe Glu Glu Ile Pro Glu
1 5 10 15

Glu Tyr Leu

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Arg Arg Arg Arg Arg Arg Arg Arg Arg Gly Asp Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Pro Leu Tyr Lys Lys Ile Ile Lys Lys Leu Leu Glu Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Gly Asp Ser
1

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Lys Pro Leu Gln Asn Phe Thr Leu Ser Phe Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Thr Lys Pro Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:37:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Arg at position 6 has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Gly Thr Lys Pro Pro Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Gly at position 1 has an N,N-dimethyl group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Cys at position 3 has an Acm group."

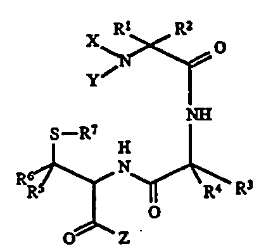
(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Arg at position 9 is unsubstituted or has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Gly Thr Cys Gly Thr Lys Pro Pro Arg
1               5
```

We claim:

1. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4} alkyl, aryl and C(O)Z;

Y is a substitutent defined by X;

X and Y may together form 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxy, oxo, C_{1-4} alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;

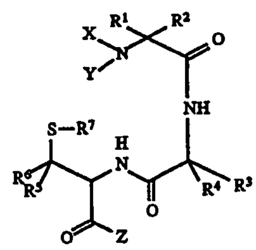
R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

R^7 is selected from H and sulfur protecting group; and

Z is selected from hydroxyl and a targeting molecule, wherein the targeting molecule is a peptide.

2. A compound according to claim 1, wherein the peptide comprises three or more amino acids.

3. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

X and Y may together form 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxy, oxo, C_{1-4} alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O) Z;

R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

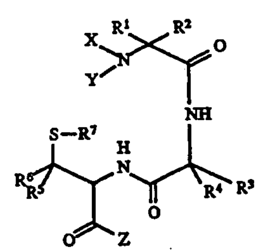
R^7 is selected from H and sulfur protecting group; and

Z is selected from hydroxyl and a targeting molecule,

in a form complexed with a metal radionuclide or an oxide or nitride thereof.

4. A compound according to claim 3, in a form complexed with ^{99m}Tc or oxide thereof.

5. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4} alkyl, aryl and C(O)Z;

Y is a substituent defined by X;

X and Y may together form 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxy, oxo, C_{1-4} alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O) Z;

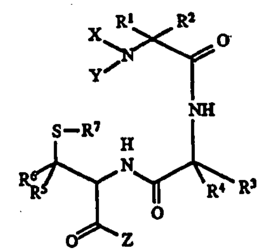
R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

R^7 is selected from H and sulfur protecting group; and

Z is selected from hydroxyl and a targeting molecule,

in a form complexed with a metal radionuclide or an oxide or nitride thereof.

6. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

X and Y may together form 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxy, oxo, C_{1-4} alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;

R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

R^7 is selected from H and sulfur protecting group; and

Z is a targeting molecule, wherein the targeting molecule is a peptide,

in a form complexed with a metal radionuclide or an oxide or nitride thereof.

7. A compound according to claim 6, in a form complexed with ^{99m}Tc or oxide thereof.

8. A compound according to claim 6, wherein the targeting molecule is a peptide comprising three or more amino acid residues.

9. A compound according to claim 8, in a form complexed with ^{99m}Tc or oxide thereof.

10. A compound according to claim 6, wherein the targeting molecule is a peptide comprising the sequence TKPPR [SEQ ID NO:36].

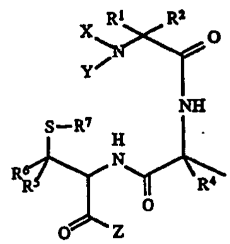
11. A compound according to claim 10, in a form complexed with ^{99m}Tc or oxide thereof.

12. A compound N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg-OH [SEQ ID NO:38],

in a form complexed with a metal radionuclide or an oxide or nitride thereof.

13. A compound according to claim 12, in a form complexed with ^{99m}Tc or oxide thereof.

14. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4} alkyl, aryl and C(O)Z;

Y is a substituent defined by X;

X and Y may together form 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carbon, oxo, C_{1-4} alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;

R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4} alkyl; C_{1-4} alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

R^7 is selected from H and sulfur protecting group; and

Z is a targeting molecule, wherein the targeting molecule is a peptide,

in a form complexed with a metal radionuclide or an oxide or nitride thereof.

15. A compound according to claim 14, wherein the peptide comprises three or more amino acid residues.

16. A compound N,N-dimethylGly-Ser-Cys (Acre)-Gly-Thr-Lys-Pro-Pro-Arg-OH [SEQ ID NO: 1].

17. The compound according to claim 16, in a form complexed with a metal radionuclide or an oxide or nitride thereof.

18. The compound according to claim 17, wherein said metal radionuclide is ^{99m}Tc.

* * * * *

B

US005780006A

United States Patent [19]

Pollak et al.

[11] Patent Number: 5,780,006

[45] Date of Patent: *Jul. 14, 1998

[54] PEPTIDE DERIVED RADIONUCLIDE CHELATORS

[75] Inventors: Alfred Pollak; Anne Goodbody, both of Toronto, Canada

[73] Assignee: Resolution Pharmaceuticals Inc., Mississauga, Canada

[*] Notice: The portion of the term of this patent subsequent to Jul. 22, 2014, has been disclaimed.

[21] Appl. No.: 703,988

[22] Filed: Aug. 28, 1996

Related U.S. Application Data

[62] Division of Ser. No. 279,155, Jul. 22, 1994, Pat. No. 5,662,885.

[51] Int. Cl.[6] A61K 51/00; A61M 36/14

[52] U.S. Cl. 424/1.69; 424/1.65; 424/1.11; 534/14; 530/300; 530/328; 530/329; 530/330

[58] Field of Search 424/1.11, 1.65, 424/1.69; 530/300, 324–330; 534/7, 10–16; 540/450; 544/63, 224; 546/152, 184, 249, 250; 548/100, 215, 300.1, 400

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,480,970	1/1996	Pollak et al.	530/328
5,569,745	10/1996	Goodbody et al.	530/328
5,662,885	9/1997	Pollak et al.	424/1.69

FOREIGN PATENT DOCUMENTS

0250013	12/1987	European Pat. Off. .
9312819	7/1993	WIPO .
9513832	5/1995	WIPO .
9522996	8/1995	WIPO .

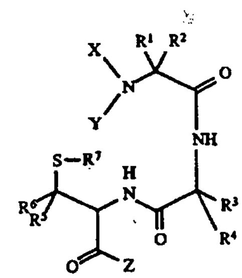
OTHER PUBLICATIONS

Pollack et al (1994), Journal of Nuclear Medicine, vol. 35, No. 5, pp. Abstract No. 171, "Imaging Inflammation with Novel Peptdic Technetium-99m Chelators Linked to a Chemotectic Peptide".

Primary Examiner—John Kight
Assistant Examiner—Dameron Jones
Attorney, Agent, or Firm—Nikaido Marmelstein Murray & Oram LLP

[57] **ABSTRACT**

For use in imaging sites of diagnostic interest within the body, the present invention provides radionuclide chelators, optionally coupled to targeting molecules such as peptides of the formula:



22 Claims, 1 Drawing Sheet

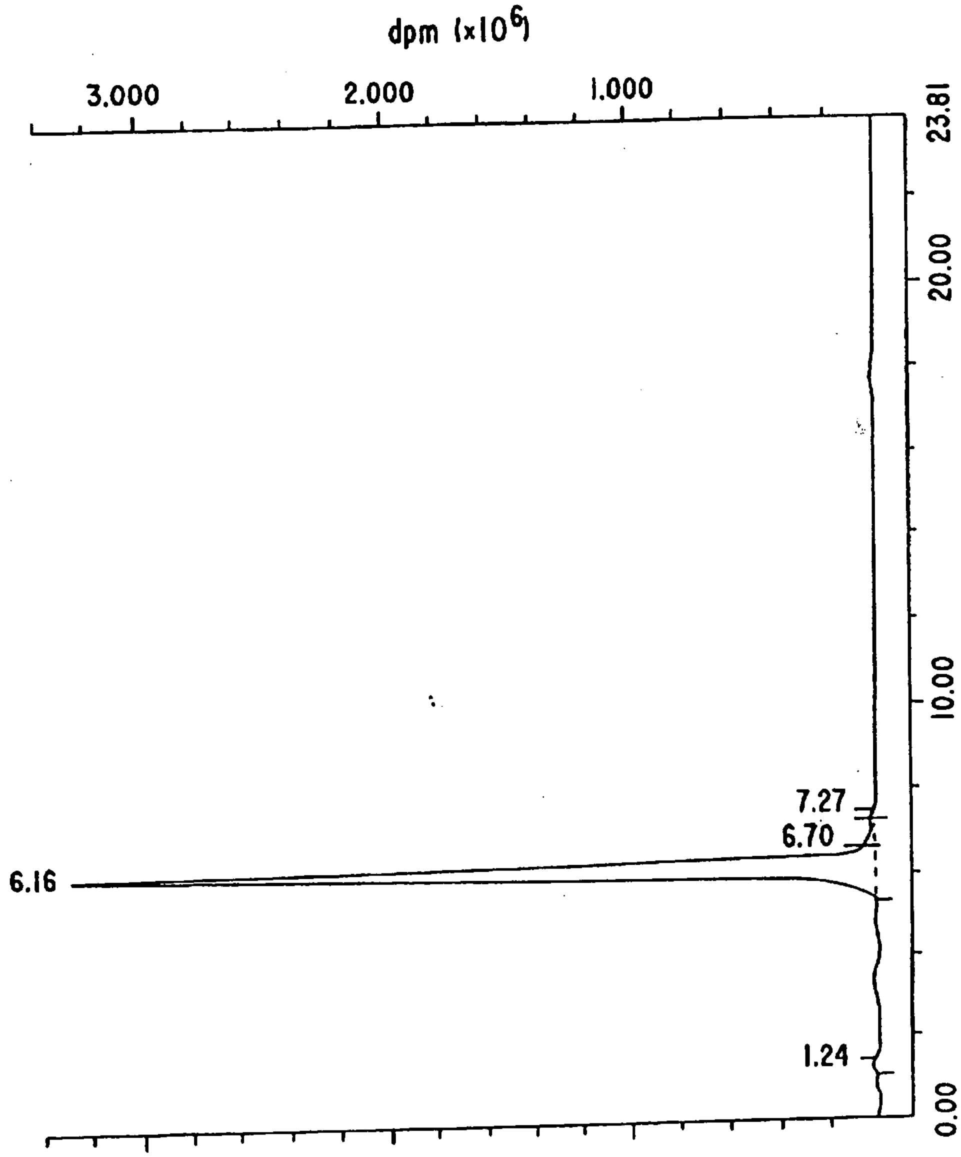


Fig. 1

PEPTIDE DERIVED RADIONUCLIDE CHELATORS

This is a division. of application Ser. No. 08/279.155 filed Jul. 22. 1994 now U.S. Pat. No. 5.662.885.

FIELD OF THE INVENTION

This invention is in the field of diagnostic imaging. and relates to chemical chelators useful in the radiolabeling of agents that target tissues of diagnostic interest.

BACKGROUND TO THE INVENTION

The art of diagnostic imaging exploits contrasting agents that in binding or localizing site selectively within the body. help to resolve the image of diagnostic Interest. ^{67}Gallum salts. for example. have an affinity for tumours and Infected tissue and. with the aid of scanning tomography. can reveal afflicted body regions to the physician. Other contrasting agents Include the metal radionuclides such as ^{99m}Technetium and $^{186/188}$rhenium. and these have been used to label targeting molecules. such as proteins. peptides and antibodies that localize at desired regions of the human body.

As targeting agents. proteins and other macromolecules can offer the tissue specificity required for diagnostic accuracy; yet labeling of these agents with metal radionuclides is made difficult by their physical structure. Particularly. protein and peptide targeting agents present numerous sites at which radionuclide binding can occur. resulting in a product that is labeled heterogeneously. Also. and despite their possibly large size. proteins rarely present the structural configuration most appropriate for high affinity radionuclide binding. i.e. a region Incorporating four or more donor atoms that form five-membered rings. As a result. radionuclides are bound typically at the more abundant low-affinity sites. forming unstable complexes.

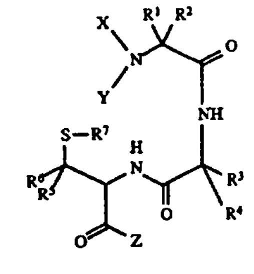
To deal with the problem of low affinity binding. Paik et al (Nucl Med Biol 1985. 12:3) proposed a method whereby labeling of antibodies is performed In the presence of excess DPTA (diaminetrimethylenepentaacetic acid). to mask the low affinity binding sites. While the problem of low affinity binding is alleviated by this method. actual binding of the radionuclide. in this case technetium. was consequently also very low. The direct labeling of proteins having a high proportion of cysteine residues also has been demonstrated (Dean et al; WO 92/13.572). This approach exploits thiol groups of cysteine residues as high-affinity sites for radionuclide binding. and is necessarily limited in application to those targeting agents having the required thiol structure.

A promising alternative to the direct labeling of targeting agents is an indirect approach. in which targeting agent and radionuclide are coupled using a chelating agent. Candidates for use as chelators are those compounds that bind tightly to the chosen metal radionuclide and also have a reactive functional group for conjugation with the targeting molecule. For use in labeling peptide and protein-based targeting agents. the chelator is ideally also peptide-based. so that the cholator-targeting molecule conjugate can be synthesized in toto using peptide synthesis techniques. For utility in diagnostic Imaging. the chelator desirably has characteristics appropriate for its in vivo use. such as blood and renal clearance and extravascular diffusibility.

SUMMARY OF THE INVENTION

The present invention provides chelators that bind diagnostically useful metal radionuclides. and can be coupled to targeting agents capable of localizing at body sites of diagnostic and therapeutic interest. The chelators of the present Invention are peptide analogs designed structurally to present an N_3S configuration capable of binding oxo. dioxo and nitrido ions of ^{99}technetium and $^{188/188}$rhenium.

More particularly. and according to one aspect of the invention. there are provided metal radionuclide chelators of the formula:



(I)

wherein

- X is a linear or branched. saturated or unsaturated C_{1-4}alkyl chain that is optionally interrupted by one or two heteroatoms selected from N. O and S; and is optionally substituted by at least one group selected from halogen. hydroxyl. amino. carboxyl. C_{1-4}alkyl. aryl and C(O)Z;
- Y is H or a substituent defined by X;
- X and Y may together form a 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen. hydroxyl. amino. carboxyl. oxo. C_{1-4}alkyl. aryl and C(O)Z;
- R^1 through R^4 are selected independently from H; carboxyl; C_{1-4}alkyl; C_{1-4}alkyl substituted with a group selected from hydroxyl. amino. sulfhydryl. halogen. carboxyl. C_{1-4}alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;
- R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4}alkyl; C_{1-4}alkyl substituted by hydroxyl. carboxyl or amino; and C(O)Z;
- R^7 is selected from H and a sulfur protecting group; and
- Z is selected from hydroxyl and a targeting molecule.

According to another aspect of the invention. the chelators of the above formula are provided in a form having the metal radionuclide complexed therewith.

In another aspect of the invention. there is provided a conjugate in which the chelator is provided in a form coupled to a diagnostically useful targeting molecule. and optionally in combination with a complexed metal radionuclide. for imaging use.

In another aspect of the invention. there is provided a method of Imaging sites of diagnostic Interest in which a conjugate of the invention is first administered as a radionuclide complex to a patient; and then the location of the radionuclide is detected using imaging means.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an HPLC analysis of conjugate N.N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg-OH |SEQ ID NO:9:| labeled with ^{99m}Tc.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides metal radionuclide chelators that when coupled to a targeting molecule are useful for deliv-

ering a radionuclide to a body site of therapeutic or diagnostic interest. As illustrated in the above formula, the chelators are peptidic compounds that present an N_3S configuration in which the radionuclide is complexed.

Terms defining the variables R^1–R^7, X, Y and Z as used hereinabove have the following meanings:

"alkyl" refers to a straight or branched C_{1-4} chain;

"aryl" refers to aromatic and heteroaromatic rings;

"halogen" refers to F, Cl and Br;

"sulfur protecting group" refers to a chemical group that inhibits oxidation of a thiol group, which includes those that are cleaved upon chelation of the metal. Suitable sulfur protecting groups include known alkyl, aryl, acyl, alkanoyl, aryloyl, mercaptoacyl and organothio groups.

In preferred embodiments of the invention, the chelators conform to the above formula in which:

R^1 through R^4 are selected independently from H; and a hydroxy-substituted C_{1-4}alkyl group such as hydroxymethyl and 1-hydroxyethyl;

R^5 and R^6 are selected independently from H and C_{1-4}alkyl, and are preferably both H;

R^7 is a hydrogen atom or a sulfur protecting group and is most preferably acetamidomethyl;

X is a C_{1-4}alkyl chain, preferably methyl or ethyl; or is a C_{1-4}alkyl chain substituted with an aryl group, preferably benzyl;

Y is H or a substituent defined by X; and is preferably methyl or ethyl and most preferably the same as X;

Z is OH or a targeting molecule, and is preferably a peptide targeting molecule.

Specific chelators of the invention include:

N,N-dimethylGly-Ser-Cys(Acm)-Z; and

N,N-dimethylGly-Thr-Cys(Acm)-Z-OH.

In the case where the substituents represented by X and Y together with the adjacent nitrogen atom form a hetero ring, such a ring may be a 5-to 8-membered, saturated ring, for example pyrrolidine, piperidine, 1-azacycloheptane and 1-azacyclooctane. Unsaturated rings formed by X and Y Include pyrrole and 4H-pyridine while it is understood that the coordinating nitrogen of the ring is necessarily trivalent and cannot form a double bond to an adjacent atom. The heterocycle formed by X and Y may also incorporate one or two additional heteroatoms selected from N, O and S. Rings having additional heteroatoms include but are not limited to 1-imidazole, pyrazole, piperazine, morpholine and thiomorpholine. The ring formed by X and Y may also be substituted with one or more and preferably less than three groups selected from halogen, hydroxyl, carboxyl, oxo, C_{1-4}alkyl and aryl, for example to form 4-oxo-1-piperidine, 4-oxo-1-pyrrolidine and 4-hydroxy-1-piperidine.

For diagnostic imaging purposes, the chelator pro se may be used in a form complexed with a metal radionuclide. Suitable radionuclides include technetium and rhenium in their various forms such as ReO^{3+}, ReO_2^{+}, $^{99m}TcO_2^{+}$ and most preferably $^{99m}TcO^{3+}$. Desirably and according to a preferred aspect of the invention, the chelator is coupled to a targeting molecule represented by Z in the above formula to form a conjugate that serves to deliver a chelated radionuclide to a desired location in a mammal. Examples of targeting molecules suitable for coupling to the chelator include, but are not limited to, steroids, proteins, peptides, antibodies, nucleotides and saccharides. Preferred targeting molecules include proteins and peptides, particularly those capable of binding with specificity to cell surface receptors

characteristic of a particular pathology. For instance, disease states associated with over-expression of particular protein receptors can be imaged by labeling that protein or a receptor binding fragment thereof coupled to a chelator of invention. Most preferably targeting molecules are peptides capable of specifically binding to target sites and have three or more amino acid residues. Targeting peptides useful to image certain medical conditions and tissues are noted below:

for atherosclerotic plaque:

YRALVDTLK [SEQ ID NO:2] RALVDTLK [SEQ ID NO:3]

RALVDTLKFVTQAEGAK [SEQ ID NO:4] YAKFRETLEDTRDRMY [SEQ ID NO:5]

AKFRETLETTRDRMY [SEQ ID NO:6] AALDLNAVANKIADFEL [SEQ ID NO:7]

YAALDLNAVANKIADFEL [SEQ ID NO:8] YRALVDTLKFVTEQAKGA [SEQ ID NO:9]

RALVDTLKFVTEQAKGA [SEQ ID NO:10] YRALVDTEFKVKQEAGAK [SEQ ID NO:11]

RALVDTEFKVKQEAGAK [SEQ ID NO:12] YRALVDTLKFVTQAEGAK [SEQ ID NO:13]

for infections and atherosclerotic plaque:

VGVAPGVGVAPGVGVAPG [SEQ ID NO:14] formyl.Nleu.LF.Nleu.YK [SEQ ID NO:15]

VPGVGVPGVGVPGVGVPGVG [SEQ ID NO:16] formylMIFL [SEQ ID NO:17]

formylMLFK [SEQ ID NO:18] formyl MLFI [SEQ ID NO:19]

formylMFIL [SEQ ID NO:20] formylMFLI [SEQ ID NO:21]

formylMLIF [SEQ ID NO:22] formylMILF [SEQ ID NO:23]

formylTKPR [SEQ ID NO:24] VGVAPG [SEQ ID NO:25]

formylMLF YIGSR [SEQ ID NO:26]

CH_2CO.YIGSRC [SEQ ID NO:27]

for thrombus:

NDGDFEEIPEYLCQ [SEQ ID NO:28] NDGDFEEIFEEY(SO_3Na)LQ [SEQ ID NO:29]

GPRG [SEQ ID NO:30]

for platelets:

D-Phe.PRPGGGGNGDFEEIPEEYL [SEQ ID NO:31] RRRRRRRRRGDV [SEQ ID NO:32]

PLYKKIIKKLLES [SEQ ID NO:33] RGD

RGDS [SEQ ID NO:34]

for amyloid plaque (Alzheimer's disease):

EKPLQNFTLSFR [SEQ ID NO:35]

For connection to the chelator, a targeting molecule may comprise a "spacer" that serves to create a physical separation between the chelator and the targeting molecule. A spacer may be an alkyl chain that is derivatized for coupling to the chelator. In the case where the targeting molecule is a peptide, the spacer may suitably be one or more amino acid residues. Preferably, peptidic targeting molecules incorporate spacers of from 1 to 5 amino acids such having chemically inert α-carbon side chains, such as glycine or β-alanine residues.

A targeting molecule may be coupled to a chelator of the invention at various sites including R^1 to R^8, X, Y and Z as well as a ring formed by X and Y. Coupling may be achieved by reacting a group present on the targeting molecule that is reactive with a substituent on the chelator to form a linkage.

For example, peptide targeting molecules having a free amino group, such as an N-terminus or an ε-amino-lysine group may be reacted with a carboxyl group on the chelator to form an amide linkage. Alternatively, the C-terminus of the peptide targeting molecule may be reacted with an amino substituent on the chelator. In a preferred embodiment, targeting molecules are coupled to chelators of formula (I) at substituent Z by an amide linkage such as a peptide bond. For example, the N-terminus amino group of a peptide targeting molecule is reacted with a carboxyl group at Z. Targeting molecules other than peptides may be coupled to chelators of the invention in a similar manner provided that a group suitable for coupling to the chelator is present. In the instance that a suitable group is not present, the targeting molecule may be chemically derivatized to present such a group. When more than one reactive group is present on the chelator or targeting molecule, it is desirable to block all but the particular group for coupling with an appropriate blocking agent in order to achieve a single conjugate species. For example, free carboxyl groups may be protected by forming esters such as a t-butyl ester which can be removed with TFA. Free amino groups may be protected with a blocking group such as FMOC which may be subsequently removed with piperidine.

In a particular embodiment of the Invention, imaging in vivo sites of focal inflammation is accomplished using a conjugate In which the targeting molecule is a chemotactic peptide comprising the amino acid sequence Thr-Lys-Pro-Pro-Lrys (TKPPR). It has been found that this peptide binds particularly well to leukocytes receptors. Targeting peptides can be spaced from the chelator by additional amino acid residues, preferably glycine, provided the peptide retains its localizing activity. In a particular embodiment, the peptide TKPPR is coupled to substituent Z of chelators according to formula (I) by a Gly residue.

Peptide-based targeting molecules can be made, either per se or as a conjugate with a chelator, using various established techniques. Because it is amenable to solid phase synthesis, employing alternating FMOC protection and deprotection is the preferred method of making short peptides. Recombinant DNA technology is preferred for producing proteins and long fragments thereof. In a particular embodiment, peptide-chelator conjugates are prepared by solid-phase peptide synthesis methods, which involve the stepwise addition of amino acid residues to a growing peptide chain that is linked to an insoluble (solid) support or matrix, such as polystyrene. The C-terminal residue of the targeting peptide is first anchored to a commercially available support with its amino group protected with an N-protecting agent such as a fluorenylmethoxycarbonyl (FMOC) group. Typically, the support is obtained with the C-terminal residue preloaded in protected form. The amino protecting group is removed with suitable deprotecting agents such as piperidine and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclocarbodiimide (DCC). Upon formation of a peptide bond, the reagents are washed from the support. Once the targeting peptide chain is synthesized, the first residue of the chelator ie. S-acetamidomethyl protected cysteine is added to the N-terminus. The final residue of the chelator is a derivatized amino acid residue that conforms to the formula (X)(Y)N—C(R^1)(R^2)—CO— wherein X, Y, R^1 and R^2 have the meaning previously defined. The final residue, for example dimethyl-glycine or sarcosine, may be commercially obtained or may be synthesized. The completed conjugate is cleaved from the support with a suitable reagent such as trifluoroacetic acid (TFA).

It will be appreciated that all substituents R^1 through R^4 according to the invention are side chains of naturally occurring or derivatized amino acids Including D-amino acids and are commercially available and compatible with solid phase synthesis techniques. Derivatized amino acid residues that are not commercially available may be incorporated in chelators of the invention by synthesizing them according to established organic chemistry techniques and inserting at the appropriate stage of solid phase peptide synthesis previously described. Similarly when substituents R^5 and R^6 are other than H, a derivatized cysteine amino acid residue is utilized In the peptide synthesis. For example, the commercially available residue penecillamine is incorporated when R^5 and R^6 are both methyl.

Various substituents at X and Y may be introduced in chelators of the invention by incorporating as the final residue of solid phase synthesis a derivatized amino acid according to the formula (X)(Y)N—C(R^1)(R^2)—C(O)—OH wherein X, Y, R^1 and R^2 have the meaning previously described. Amino acids having N-terminal amino substituents according to X and Y may be synthesized according to established organic chemistry procedures and techniques. For example, when X and Y are both dibenzyl substituents the corresponding dibenzylglycine residue may be synthesized by reacting commercially available reagents bromoacetic acid and dibenzylamine in a suitable solvent such as dichloromethane and then heating. Other amines may be employed in the reaction in place of dibenzylamine such as diisopropylamine to give the corresponding diisopropylglycine. Similarly cyclic amines such as piperidine and morpholine in place of dibenzylamine will give the corresponding piperidylglycine and morpholinylglycine residues.

In a most preferred embodiment of the invention a peptide-chelator conjugate is prepared on a solid support and has the structure of formula (I) wherein the targeting molecule is a peptide having a sequence Gly-Thr-Lys-Pro-Pro-Arg-OH|SEQ ID NO:37|R^1, R^2, R^3, R^5 and R^6 are H; R^4 is hydroxymethyl or 1-hydroxyethyl and R^7 is acetamidomethyl.

Incorporation of the selected radionuclide within the chelator can be achieved by various established methods. For example the following general procedure may be used. A chelator solution is formed initially by dissolving the chelator in aqueous alcohol eg. ethanol-water 1:1. Oxygen is removed for example by degassing with N_2, then sodium hydroxide is added to remove the thiol protecting group. The solution is again purged of oxygen and heated on a water bath to hydrolyze the thiol protecting group, and the solution is then neutralized with an organic acid such as acetic acid (pH 6.0–6.5). In the labeling step, sodium pertechnetate is added to a chelator solution with an amount of stannous chloride sufficient to reduce the technetium. The solution is mixed and left to react at room temperature and then heated on a water bath. In an alternative method, labeling can be accomplished with the chelator solution adjusted to pH 8. At this higher pH, pertechnetate may be replaced with a solution containing technetium complexed with labile ligands suitable for ligand exchange reactions with the desired chelator. Suitable ligands include tartarate, citrate and heptagluconate. Stannous chloride may be replaced with sodium dithionite as the reducing agent if the chelating solution is alternatively adjusted to a still higher pH of 12–13 for the labeling step. The chelators of the present invention can be coupled to a targeting molecule prior to labeling with the radionuclide, a process referred to as the "bifunctional chelate" method. An alternative approach is the "prelabeled ligand" method in which the chelator is first labeled with a radionuclide and is then coupled to the targeting molecule.

The labeled chelator may be separated from contaminants $^{99m}TcO_4^-$ and colloidal $^{99m}TcO_2$ chromatographically, e.g., with a C-18 Sep Pak column activated with ethanol followed by dilute HCl. Eluting with dilute HCl separates the $^{99m}TcO_4^-$, and eluting with EtOH-saline 1:1 brings off the chelator while colloidal $^{99m}TcO_2$ remains on the column.

When coupled to a targeting molecule and labeled with a diagnostically useful metal, chelators of the present invention can be used to detect pathological conditions by techniques common in the art of diagnostic imaging. A chelator-targeting molecule conjugate labeled with a radionuclide metal such as technetium may be administered to a mammal intralymphatically, intraperitoneally and preferably intravenously in a pharmaceutically acceptable solution such as saline or blood plasma medium. The amount of labeled conjugate administered is dependent upon the toxicity profile of the chosen targeting molecule as well as the profile of the metal and is typically in the range of about 0.01 to 100 and preferably 10 to 50mCi per 70 Kg host. Localization of the metal in vito is tracked by standard scintigraphic techniques at an appropriate time subsequent to its administration. The time at which an image may be obtained will depend upon the profile of the targeting molecule, for example most peptides will localize rapidly allowing for an Image to be obtained within 3 hours and often within 1 hour. In a particular embodiment, chelators of the invention coupled to a peptide targeting molecule GTKPPR in a saline solution are administered by Intravenous injection to image sites of focal inflammation.

The following examples are presented to illustrate certain embodiments of the present invention.

EXAMPLE 1

Preparation of Peptide-Chelator Conjugates

N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:1] and

N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO:38]

The title conjugates were prepared as a single peptide chain by solid phase peptide synthesis using FMOC chemistry on an FMOC-arginine preloaded 2-methoxy-4-alkoxybenzyl alcohol resin (Sasrin Resin, Bachem Biosciences In., Philadelphia) with an Applied Biosystems 433A peptide synthesizer (Foster City, Calif.). Derivatized amino acid residues S-acetamidomethyl-cysteine (Bachem) and N,N-dimethylglycine (Sigma Chemical Company, St. Louis, Mo.) were incorporated at the appropriate step of chain elongation.

Upon addition of the final residue N,N-dimethylGly, the peptide-resin was dried under vacuum overnight and cleavage of the peptide from the resin was achieved by mixing a cooled solution of 9.5 mL trifluoroacetic acid (TFA), 0.5 mL water, 0.5 mL thioanisole and 0.25 mL 2-ethanedithiol (1 mL per 100 mg of peptide-resin) with the peptide-resin for 1.5 to 2 hours at room temperature. The resin was removed by filtration and washed with 1–3 mL of TFA to obtain 6–8 mL of a clear yellow liquid. This liquid was slowly dropped into 30–35 mL of cold tert-butyl ether in a 50 mL conical polypropylene centrifuge tube forming a white precipitate. The precipitate was centrifuged at 7000 rpm, 0° C. for 5 minutes (Sorvall RT6000, Dupont), decanted and washed two more times with tert-butyl ether. Following drying under vacuum the precipitate was dissolved in water. The solution was frozen in acetone-dry ice and lyophilized overnight. The resulting white powder was dissolved in water, filtered through a 0.45 μm syringe filter (Gelman Acrodisc 3 CR PTFE), and purified by reversed-phase HPLC (Beckman System Gold) with a C18 column (Waters RCM 25×10) using 1% TFA in water as buffer A and 1% TFA in acetonitrile as buffer B. The column was equilibrated with 100:0 buffer A:buffer B and eluted with a linear gradient in 25 minutes at 1 mL/min to 50% buffer B. Fractions were reanalysed on the HPLC and pooled according to matching profiles. The pure fractions were frozen in acetone-dry ice and lyophilized 12 hours to give a white powder.

EXAMPLE 2

Labeling of Peptide-Chelator Conjugates

N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-ProPro-Arg [SEQ ID NO:1] and

N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg [SEQ ID NO: 38]

The conjugates of example 1 were reconstituted (200 μL, 1 mg/mL saline) and then injected into 3 mL vacutainers with 100 μL pertechnetate (10 mCi) and 100 μL stannous gluconate (50 μg stannous chloride and 1 mg sodium gluconate). The tubes were placed in boiling water bath for 12 minutes and then filtered through a Whatman PVDF syringe filter to collect the labeled conjugate solutions which were further diluted with saline to prepare injectable solutions (2 Mbq/mL). The conjugates were isolated by HPLC (Beckman) from a (20 μL) sample (before dilution) to determine the labeling yield by measuring radioactivity. Each conjugate gave a single peak and greater than 94% labeling yield. 24 hours after labeling, N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg[SEQ ID NO:1] was reanalyzed on HPLC. No degradation or radiolysis products were observed.

EXAMPLE 3

In vivo Imaging and Biodistribution of Conjugates

Rat inflammation studies were performed as follows. 2 male Wistar rats (Charles River, 200–250 g) were injected intramuscularly with (25 mg) zymosan, a yeast cell wall suspension, into their left hindlegs 24 hours before imaging. Focal inflammation in the leg was visually detectable after 1 day. 1 mg (ca. 0.7 μMol) of the chelator-peptide conjugate was dissolved in 50 μL of dimethylsulfoxide and added to an ethanol-water mixture (1:1, 200 μL). An aliquot of Tc-99 m tartarate (ca. 400 MBq) was added and transchelation allowed to proceed for 20 min. at 100° C. The Tc-99 m labeled conjugate was purified by elution through a Sep Pak cartridge and then diluted with saline to prepare an injectable formulation (200 μL) containing about 100 μCi (3.7 MBq) of activity.

The rats were anaesthetized with somnitol (40 to 50 mg/kg), and the labeled conjugate solution (200 μL) was injected intravenously via the tail vein. Serial whole-body scintigrams were acquired at 30 minutes. The rats were then killed with anaesthesia overdose and samples of organs, urine, blood, inflamed muscle (left leg) and non-inflamed muscle (right leg) and inflammatory exudate (fluid) were weighed and counted in either a well-type gamma counter or in a gamma dose calibrator depending upon the organ. The dose calculations were made based on the assumption that the blood volume constituted 8% of body weight. The results of the conjugates represented in the table below are averages for two rats and are corrected for the residual dose in the tail.

Both conjugates gave excellent scintigraphic images in comparison to other known inflammation imaging agents such as Ga-67, ^{99m}Tc-IgG, ^{111}In-WBC and ^{99m}Tc-Nanocoll which is indicated by the high target to background ratios (inflamed:uninflamed muscle) observed. The conjugates imaged much more rapidly than the known agents and exhibited superior biodistribution. Also, non-target organs such as liver and GI tract showed low accumulation.

Imaging Agent	Inflam:Uninfl	Fluid:Blood	Urine (% dose)	Liver (% dose)	GI Tract (% dose)
N,N-dimethylGly—Ser—Cys(Acm)—GTKPPR—OH [SER ID NO: 1]	5.3	1.9	63.5	2.4	2.8
N,N-dimethylGly—Thr—Cys(Acm)—GTKPPR—OH [SER ID NO: 38]	4.6	1.6	68.5	2.4	2.5
^{67}Ga	2.5	0.1	5.5	26.5	8.4
^{99m}TC—IgG	2.8	0.03	1.2	17.6	0.7
^{111}In—WBC	1.5	0.1	0.2	36.9	3.6
^{99m}Tc—Nanocoll	3.3	0.2	0.8	66.7	2.1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 38

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Gly at position 1 has an N,N- dimethyl group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Cys at position 3 has an Acm group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Arg at position 9 is unsubstituted or has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gly Ser Cys Gly Thr Lys Pro Pro Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Tyr Arg Ala Leu Val Asp Thr Leu Lys
1               5
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Ala Leu Val Asp Thr Leu Lys
1   5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Gln Ala Glu
1   5   10

Gly Ala Lys
15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Ala Lys Phe Arg Glu Thr Leu Glu Asp Thr Arg Asp Arg
1   5   10

Met Tyr
15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Lys Phe Arg Glu Thr Leu Glu Asp Thr Arg Asp Arg Met
1   5   10

Tyr
15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

-continued

Ala Ala Leu Asp Leu Asn Ala Val Ala Asn Lys Ile Ala Asp
1 5 10

Phe Glu Leu
15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Tyr Ala Ala Leu Asp Leu Asn Ala Val Ala Asn Lys Ile Ala
1 5 10

Asp Phe Glu Leu
15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Glu Gln
1 5 10

Ala Lys Gly Ala
15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Glu Gln Ala
1 5 10

Lys Gly Ala
15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Tyr Arg Ala Leu Val Asp Thr Glu Phe Lys Val Lys Gln Glu
1 5 10

Ala Gly Ala Lys

15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Arg Ala Leu Val Asp Thr Glu Phe Lys Val Lys Gln Glu Ala
1 5 10

Gly Ala Lys
15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Tyr Arg Ala Leu Val Asp Thr Leu Lys Phe Val Thr Gln Ala
1 5 10

Glu Gly Ala Lys
15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val Gly Val Ala Pro Gly Val Gly Val Ala Pro Gly Val Gly
1 5 10

Val Ala Pro Gly
15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Leu at position 1 is formylLNorLeu."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Leu at position 4 is

NorLeu."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Leu Phe Leu Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
1 5 10

Gly Val Pro Gly Val Gly
15 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Ile Phe Leu
1

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Leu Phe Lys
1

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Leu Phe Ile
1

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Phe Ile Leu
1

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Phe Leu Ile
1

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Met at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Leu Ile Phe
1

(2) INFORMATION FOR SEQ ID NO:23:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ile Leu Phe
1

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Thr at position 1 has a formyl group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Lys Pro Arg
1

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Val Gly Val Ala Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Tyr Ile Gly Ser Arg
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Tyr at position 1 has a CH2CO group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Tyr Ile Gly Ser Arg Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Asn Asp Gly Asp Phe Glu Glu Ile Pro Glu Glu Tyr Leu Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Asn Asp Gly Asp Phe Glu Glu Ile Pro Glu Glu Tyr Leu Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Gly Pro Arg Gly
1
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Pro at position 1 has a D-Phe group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Pro Arg Pro Gly Gly Gly Gly Asn Gly Asp Phe Glu Glu Ile
1               5                   10
```

Pro Glu Gln Tyr Leu
15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Arg Arg Arg Arg Arg Arg Arg Arg Arg Gly Asp Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Pro Leu Tyr Lys Lys Ile Ile Lys Lys Leu Leu Glu Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Gly Asp Ser
1

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Lys Pro Leu Gln Asn Phe Thr Leu Ser Phe Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

-continued

Thr Lys Pro Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Arg at position 6 has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Gly Thr Lys Pro Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Gly at position 1 has an N,N- dimethyl group."

(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Cys at position 3 has an Acm group."

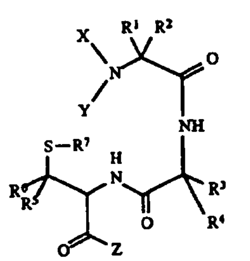
(i x) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= "Arg at position 9 is unsubstituted or has an OH group."

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Thr Cys Gly Thr Lys Pro Pro Arg
1 5

We claim:

1. A compound of the general formula:



wherein

X is a linear or branched, saturated or unsaturated C_{1-4}alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O and S; and is optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, C_{1-4}alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X; or

X or and Y together form a 5- to 8-membered saturated or unsaturated heterocyclic ring optionally substituted by at least one group selected from halogen, hydroxyl, amino, carboxyl, oxo, C_{1-4}alkyl, aryl and C(O)Z;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4}alkyl; C_{1-4}alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen,

carboxyl, C_{1-4}alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L-amino acid other than proline; and C(O)Z;

R^5 and R^6 are selected independently from H; carboxyl; amino; C_{1-4}alkyl; C_{1-4}alkyl substituted by hydroxyl, carboxyl or amino; and C(O)Z;

R^7 is selected from H and a sulfur protecting group; and

Z is selected from hydroxyl and a targeting molecule.

2. A compound according to claim 1, wherein R^1, R^2, R^4, R^5 and R^6 are hydrogen.

3. A compound according to claim 1, wherein X and Y are independently both C_{1-4}alkyl.

4. A compound according to claim 1, wherein R^3 is selected from hydroxymethyl and 1-hydroxyethyl.

5. A compound according to claim 1, wherein Y is a substituent defined by X.

6. A compound according to claim 5, wherein X and Y are both methyl.

7. A compound according to claim 5, wherein R^3 is selected from hydroxymethyl and 1-hydroxyethyl.

8. A compound according to claim 5, wherein R^1, R^2, R^4, R^5 and R^6 are hydrogen.

9. A compound according to claim 1, wherein Z is a targeting molecule.

10. A compound according to claim 9, wherein the targeting molecule is a peptide.

11. A compound according to claim 10, wherein the peptide comprises 3 or more amino acid residues.

12. A compound according to claim 11, wherein the peptide comprises the sequence TKPPR.

13. A compound according to claim 12, wherein the peptide comprises the sequence Gly-Thr-Lys-Pro-Pro-Arg-OH.

14. A compound according to claim 1, that is N,N-dimethylGly-Thr-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg-OH.

15. A method of detecting the localization of a targeting molecule within a mammal comprising the step of administering a diagnostically effective amount of a compound according to claim 1, wherein Z is the targeting molecule and said compound is in a form complexed with a metal radionuclide or an oxide or nitride thereof.

16. The method according to claim 15, wherein said metal radionuclide is ^{99m}Tc.

17. A method of imaging a site of focal inflammation within a mammal comprising the step of administering a diagnostically effective amount of a compound according to claim 10, in a form complexed with a metal radionuclide or an oxide or nitride thereof.

18. The method according to claim 17, wherein said metal radionuclide is ^{99m}Tc.

19. A method of imaging a site of focal inflammation within a mammal comprising the step of administering an effective amount of a compound according to claim 14, in a form complexed with a metal radionuclide or an oxide or nitride thereof.

20. The method according to claim 19, wherein said metal radionuclide is ^{99m}Tc.

21. A method of imaging a site of focal inflammation within a mammal comprising the step of administering an effective amount of N,N-dimethylGly-Ser-Cys(Acm)-Gly-Thr-Lys-Pro-Pro-Arg-OH in a form complexed with a metal radionuclide or an oxide or nitride thereof.

22. A method of imaging a site of focal inflammation within a mammal comprising the step of administering an effective amount of a compound according to claim 21, wherein said metal radionuclide is ^{99m}Tc.

* * * * *

C

DIII 110

USSN 08/092,3…

APPLICATION FOR UNITED STATES LETTERS PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(Case No. 92,385-C)

Inventors:

Richard T. Dean
43 King Road
Bedford, New Hampshire 03110

A Citizen of the United States of America

William McBride
110 Golfview Drive
Manchester, New Hampshire 03102

A Citizen of the United States of America

John Lister-James
25 Old Stone Way
Bedford, New Hampshire 03110

A Citizen of the United Kingdom

Assignment:

Diatech, Inc.
9 Delta Drive
Londonderry, New Hampshire 03053

Title: RADIOLABELED PEPTIDES

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

RADIOLABELED PEPTIDES

CIP 07/807062
USP 5,443,815

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to therapeutic agents and peptides, radiotherapeutic agents and peptides, radiodiagnostic agents and peptides, and methods for producing such labeled radiodiagnostic and radiotherapeutic agents. Specifically, the invention relates to cyclic peptide derivatives and analogues of somatostatin, and embodiments of such peptides labeled with gamma-radiation emitting isotopes such as technetium-99m (Tc-99m), as well as methods and kits for making, radiolabeling and using such peptides to image sites in a mammalian body. The invention also relates to peptide derivatives and analogues of somatostatin labeled with cytotoxic radioisotopes such as rhenium-186 (^{186}Re) and rhenium-188 (^{188}Re), and methods and kits for making, radiolabeling and using such peptides therapeutically in a mammalian body.

2. Description of the Prior Art

Somatostatin is a tetradecapeptide that is endogenously produced by the hypothalamus and pancreas in humans and other mammals. The peptide has the formula:

Formula I

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

[Single letter abbreviations for amino acids can be found in G. Zubay, Biochemistry (2d ed.), 1988, (MacMillan Publishing: New York), p.33]. This peptide exerts a wide variety of biological effects *in vivo*. It is known to act physiologically on the central nervous system, the hypothalamus, the pancreas, and the gastrointestinal tract.

Somatostatin inhibits the release of insulin and glucagon from the pancreas, inhibits growth hormone release from the hypothalamus, and reduces gastric secretions. Thus, somatostatin has clinical and therapeutic applications for the alleviation of a number of

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

ailments and diseases, both in humans and other animals. Native somatostatin is of limited utility, however, due to its short half-life *in vivo*, where it is rapidly degraded by peptidases. For this reason, somatostatin analogues having improved *in vivo* stability have been developed in the prior art.

Freidinger, U.S. Patent No. 4,235,886 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Coy and Murphy, U.S. Patent No. 4,485,101 disclose synthetic dodecapeptide somatostatin analogues.

Freidinger, U.S. Patent No. 4,611,054 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Nutt, U.S. Patent No. 4,612,366 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Coy *et al.*, U.S. Patent No. 4,853,371 disclose synthetic octapeptide somatostatin analogues.

Coy and Murphy, U.S. Patent No. 4,871,717 disclose synthetic heptapeptide somatostatin analogues.

Coy *et al.*, U.S. Patent No. 4,904,642 disclose synthetic octapeptide somatostatin analogues.

Taylor *et al.*, U.S. Patent No. 5,073,541 disclose a method of treating small cell lung cancer.

Brady, European Patent Application No. 83111747.8 discloses dicyclic hexapeptide somatostatin analogues useful in the treatment of a number of human diseases.

Bauer *et al.*, European Patent Application No. 85810617.2 disclose somatostatin derivatives useful in the treatment of a number of human diseases.

Eck and Moreau, European Patent Application No. 90302760.5 disclose therapeutic octapeptide somatostatin analogues.

Coy and Murphy, International Patent Application Serial No. PCT/US90/07074 disclose somatostatin analogues for therapeutic uses.

Schally *et al.*, European Patent Application Serial No. EPA 911048445.2 disclose

cyclic peptides for therapeutic use.

Bodgen and Moreau, International Patent Application Serial No. PCT/US92/01027 disclose compositions and methods for treating proliferative skin disease.

Somatostatin exerts it effects by binding to specific receptors expressed at the cell surface of cells comprising the central nervous system, the hypothalamus, the pancreas, and the gastrointestinal tract. These high-affinity somatostatin binding sites have been found to be abundantly expressed at the cell surface of most endocrine-active tumors arising from these tissues. Expression of high-affinity binding sites for somatostatin is a marker for these tumor cells, and specific binding with somatostatin can be exploited to locate and identify tumor cells *in vivo*.

Methods for radiolabeling somatostatin analogues that have been modified so as to contain a tyrosine amino acid (Tyr or Y) are known in the prior art.

Albert *et al.*, UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{123}I.

Bakker *et al.*, 1990, J. Nucl. Med. 31: 1501-1509 describe radioactive iodination of a somatostatin analog and its usefulness in detecting tumors *in vivo*.

Bakker *et al.*, 1991, J. Nucl. Med. 32: 1184-1189 teach the usefulness of radiolabeled somatostatin for radioimaging *in vivo*.

Bomanji *et al.*, 1992, J. Nucl. Med. 33: 1121-1124 describe the use of iodinated (Tyr-3) octreotide for imaging metastatic carcinoid tumors.

Alternatively, methods for radiolabeling somatostatin by covalently modifying the peptide to contain a radionuclide-chelating group have been disclosed in the prior art.

Albert *et al.*, UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{111}In *via* a chelating group bound to the amino-terminus.

Albert *et al.*, European Patent Application No. WO 91/01144 disclose radioimaging using radiolabeled peptides related to growth factors, hormones, interferons and cytokines and comprised of a specific recognition peptide covalently linked to a radionuclide chelating group.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

Albert *et al.*, European Patent Application No. 92810381.1 disclose somatostatin peptides having amino-terminally linked chelators.

Faglia *et al.*, 1991, J. Clin. Endocrinol. Metab. 73: 850-856 describe the detection of somatostatin receptors in patients.

Kwekkeboom *et al.*, 1991, J. Nucl. Med. 32: 981 Abstract #305 relates to radiolabeling somatostatin analogues with ^{111}In.

Albert *et al.*, 1991, Abstract LM10, 12th American Peptide Symposium: 1991 describe uses for ^{111}In-labeled diethylene-triaminopentaacetic acid-derivatized somatostatin analogues.

Krenning *et al.*, 1992, J. Nucl. Med. 33: 652-658 describe clinical scintigraphy using [^{111}In][DTPA]octreotide.

These methods can be readily adapted to enable detection of tumor cells *in vivo* by radioimaging, based on the expression of high affinity binding sites for somatostatin on tumor cells. Radionuclides which emit gamma radiation can be readily detected by scintigraphy after injection into a human or an animal. A variety of radionuclides are known to be useful for radioimaging, including ^{67}Ga, ^{68}Ga, ^{99m}Tc (Tc-99m), ^{111}In, ^{123}I or ^{125}I. The sensitivity of imaging methods using radioactively-labeled peptides is much higher than other techniques known in the art, since the specific binding of the radioactive peptide concentrates the radioactive signal over the cells of interest, for example, tumor cells. This is particularly important for endocrine-active gastrointestinal tumors, which are usually small, slow-growing and difficult to detect by conventional methods. Labeling with technetium-99m (Tc-99m) is advantageous because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. Tc-99m has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator. Other radionuclides have effective half-lives which are much longer (*for example*, ^{111}In, which has a half-life of 60-70 h) or are toxic (*for example*, ^{125}I). Although Tc-99m is an ideal radiolabeling reagent, it has not been widely used in the art prior to the present invention [*see*, *for example*, Lamberts, J. Nucl. Med. 32: 1189-1191 (1991)].

Somatostatin and radiolabeled somatostatin analogues can also be used therapeutically. For these applications, cytotoxic radioisotopes are advantageous, such as scandium-47, copper-

67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, astatine-211 and bismuth-212. The rhenium isotopes ^{186}Re and ^{188}Re are particularly advantageous.

updated 27 April 98

The use of chelating agents for radiolabeling proteins are known in the prior art, and methods for labeling peptides Tc-99m are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/653,012, 07/757,470, 07/807,062, 07/851,074, 07/871,282, 07/886,752, 07/893,981, 07/955,466, 07/977,628, 08/019,864, 08/044,825 and 08/073,577, and PCT International Applications PCT/US92/00757, PCT/US92/10716, PCT/US93/02320, PCT/US93/03687, PCT/US93/04794, PCT/US93/05372, and PCT/US93/06029, which are hereby incorporated by reference.

Fritzberg, U.S. Patent No. 4,444,690 describes a series of technetium-chelating agents based on 2,3-bis(mercaptoacetamido) propanoate.

Gansow *et al.*, U.S. Patent No. 4,472,509 teach methods of manufacturing and purifying Tc-99m chelate-conjugated monoclonal antibodies.

Reno and Bottino, European Patent Application 87300426.1 disclose radiolabeling antibodies with Tc-99m.

Pak *et al.*, European Patent Application No. WO 88/07382 disclose a method for labeling antibodies with Tc-99m.

Cox, International Patent Application No. PCT/US92/04559 discloses radiolabeled somatostatin derivatives containing two cysteine residues.

Rhodes, 1974, Sem. Nucl. Med. 4: 281-293 teach the labeling of human serum albumin with technetium-99m.

Khaw *et al.*, 1982, J. Nucl. Med. 23: 1011-1019 disclose methods for labeling biologically active macromolecules with Tc-99m.

Byrne and Tolman, *supra*, disclose a bifunctional thiolactone chelating agent for coupling Tc-99m to biological molecules.

Cox *et al.*, 1991, Abstract, 7th International Symposium on Radiopharmacology, p. 16, disclose the use of, Tc-99m-, ^{131}I- and ^{111}In-labeled somatostatin analogues in

radiolocalization of endocrine tumors *in vivo* by scintigraphy.

Methods for directly labeling somatostatin, derivatives of somatostatin, analogues of somatostatin or peptides that bind to the somatostatin receptor and contain at least 2 cysteine residues that form a disulfide or wherein the disulfide is reduced to the sulfhydryl form, are disclosed in co-pending U.S. Patent Application Serial No. 07/807,062, now U.S. Patent No. 5,225,180, issued July 6, 1993 which is hereby incorporated by reference.

There remains a need for synthetic (to make routine manufacture practicable and to ease regulatory acceptance) somatostatin analogues having increased *in vivo* stability, to be used therapeutically, as scintigraphic agents when radiolabeled with Tc-99m or other detectable radioisotopes for use in imaging tumors *in vivo*, and as radiotherapeutic agents when radiolabeled with a cytotoxic radioisotope such as rhenium-188. Small synthetic somatostatin analogues are provided by this invention that specifically fulfill this need.

SUMMARY OF THE INVENTION

The present invention provides somatostatin analogues that are cyclic peptides for therapeutic applications, including radiotherapeutic applications, and diagnostic applications, including radiodiagnostic applications, in particular scintigraphic imaging applications. Distinct from native somatostatin and somatostatin analogues known in the prior art, the cyclic peptides of the invention are not comprised of a disulfide bond. The invention also provides cyclic peptide reagents comprised of the cyclic peptide somatostatin analogues of the invention, wherein such peptides are covalently linked to a radiolabel-binding moiety. The invention provides such cyclic peptides, cyclic peptide reagents and radiolabeled cyclic peptide reagents that are scintigraphic imaging agents, radiodiagnostic agents and radiotherapeutic agents. Scintigraphic imaging agents of the invention comprise cyclic peptide reagents radiolabeled with a radioisotope, preferably technetium-99m. Radiotherapeutic agents of the invention comprise cyclic peptide reagents radiolabeled with a cytotoxic radioisotope, preferably rhenium-186 or rhenium-188. Methods for making and using such cyclic peptides, cyclic peptide reagents and radiolabeled embodiments thereof are also provided.

The invention provides a cyclic peptide that is a somatostatin analogue as a composition of matter comprising a somatostatin-receptor binding peptide having the formula:

Formula II

$$\begin{array}{l} R^1(CR^2)\text{-}[C(R^3R^4)]_m\text{-}CO\text{-}A^1A^2A^3B^1B^2B^3B^4C^1C^2C^3\text{-}NH\text{-}CR^{11}\text{-}X^2 \\ \quad | \\ Z \text{———} (CR^7R^8) \text{—————} (CR^5R^6)_p \end{array}$$

where R^1, R^2, R^5 and R^6 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl; R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or wherein either R^3 or R^4 is X^1; A^1 and C^3 are independently a bond or a D- or L-amino acid; A^2, A^3 and C^1 are each independently a bond or a lipophilic D- or L-amino acid; B^1 is D- or L-Phe or D- or L-Tyr or D- or L-2-naphthylalanine (Nal) or 2-aminoindan-2-carboxylic acid (Ain) or a substituted derivative thereof; B^2 is D- or L-Trp or a substituted derivative thereof; B^3 is D- or L-Lys or homolysine (Hly), 4-amino-cyclohexylalanine (Achxa), 4-aminomethylphenylalanine (Amf), S-(2-aminoethyl)cysteine (Aec), S-(3-aminopropyl)cysteine (Apc), O-(2-aminoethyl) serine (Aes), O-(3-aminopropyl)serine (Aps) or a substituted derivative thereof; B^4 is Thr, Ser, Val, Phe, Ile, Leu, 2-amino-isobutyric acid (Aib), 2-aminobutyric acid (Abu), norvaline (Nva), or norleucine (Nle); C^2 is a bond or D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or a substituted derivative thereof; X^1 is $N(R^{10})_2$, where each R^{10} is independently hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl or a hydrophilic moiety of less than about 1500 daltons; X^2 is $-COOR^9$, $-CH_2OH$, CH_2COOR^9, or $-CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or a substituted derivative thereof or a hydrophilic moiety of less than about 1500 daltons; and where m is 0,1,2 or 3 and p is 0, 1 or 2; R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 or R^8 are -COOH or $-CO.N(R^{10})_2$ or $-COOR^{12}$, or R^7 and R^8 together are an oxygen atom; R^{12} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl; Z is a sulfur atom, an oxygen atom, NR^{13}, $NR^{13}NR^{13}$, $NR^{13}.CO.NR^{13}$, SO_2, $NR^{13}SO_2$ or the moiety (S=O); and further where R^{13} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl; and where Z is NR^{13}, R^7 and R^8 are not together an oxygen. In a preferred embodiment, the X^1 moiety is an amino acid or a peptide sequence comprising 10 or fewer amino acids, or a monosaccharaide or oligosaccharide comprising 10 or fewer saccharide

units, or a poly(*N*-carboxyalkyl)amine or a poly-oxy anion and the X^2 moiety is poly(*N*-carboxyalkyl)amine or a polyoxy-anion, or an amino acid or a peptide having an amino acid sequence of no more than 10 residues (including peptides wherein the carboxyl group of the carboxyl-terminal amino acid is reduced to an alcohol), or a monosaccharide or oligosaccharide comprising 10 or fewer saccharide units. In another preferred embodiment, B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

The invention also provides a cyclic peptide that is a somatostatin analogue as a composition of matter comprising a somatostatin-receptor binding peptide having the formula:

<u>Formula III</u>

cyclo-A^4-$B^1B^2B^3B^4$-C^4-

where B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or a substituted derivative thereof; B^2 is D- or L-Trp or a substituted derivative thereof; B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or a substituted derivative thereof; B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib; C^4 is an L-amino acid comprising a sidechain having a mercapto group; and A^4 is a lipophilic D-amino acid or a lipophilic L-(α-*N*-alkyl) amino acid or L-cysteine or L-proline or a substituted derivative thereof. This moiety is a cyclic peptide moiety, where the amino terminus of the A^4 residue is covalently linked with the carboxyl terminus of the C^4 residue. In a preferred embodiment, B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

The invention also provides a cyclic peptide reagent comprising a somatostatin-receptor binding peptide having the formula:

<u>Formula II</u>

$$
\begin{array}{l}
R^1(CR^2)\text{-}[C(R^3R^4)]_m\text{-}CO\text{-}A^1A^2A^3B^1B^2B^3B^4C^1C^2C^3\text{-}NH\text{-}CR^{11}\text{-}X^2 \\
\quad | \\
\;Z \text{ --- } (CR^7R^8) \text{ ------------ } (CR^5R^6)_p
\end{array}
$$

where R^1, R^2, R^5 and R^6 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl; R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or wherein either R^3 or R^4 is X^1; A^1 and C^3 are independently a bond

or a D- or L-amino acid; A^2, A^3 and C^1 are each independently a bond or a lipophilic D- or L-amino acid; B^1 is D- or L-Phe or D- or L-Tyr or D- or L-2-naphthylalanine (Nal) or 2-aminoindan-2-carboxylic acid (Ain) or a substituted derivative thereof; B^2 is D- or L-Trp or a substituted derivative thereof; B^3 is D- or L-Lys or homolysine (Hly), 4-amino-cyclohexylalanine (Achxa), 4-aminomethylphenylalanine (Amf), S-(2-aminoethyl)cysteine (Aec), S-(3-aminopropyl)cysteine (Apc), O-(2-aminoethyl) serine (Aes), O-(3-aminopropyl)serine (Aps) or a substituted derivative thereof; B^4 is Thr, Ser, Val, Phe, Ile, Leu, 2-amino-isobutyric acid (Aib), 2-aminobutyric acid (Abu), norvaline (Nva), or norleucine (Nle); C^2 is a bond or D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or a substituted derivative thereof; X^1 is $N(R^{10})_2$, where each R^{10} is independently hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl or substituted with a hydrophilic moiety of less than about 1500 daltons; X^2 is $-COOR^9$, $-CH_2OH$, CH_2COOR^9, or $-CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or a substituted derivative thereof or substituted with a hydrophilic moiety of less than about 1500 daltons; and where m is 0,1,2 or 3 and p is 0, 1 or 2; R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 or R^8 are -COOH or $-CO.N(R^{10})_2$ or $-COOR^{12}$, or R^7 and R^8 together are an oxygen atom; R^{12} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl; Z is a bond, a sulfur atom, an oxygen atom, NR^{13}, $NR^{13}NR^{13}$, $NR^{13}.CO.NR^{13}$, SO_2, $NR^{13}SO_2$ or the moiety (S=O); and further where R^{13} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl; and where Z is NR^{13}, R^7 and R^8 are not together an oxygen. In a preferred embodiment, the X^1 moiety is an amino acid or a peptide sequence comprising 10 or fewer amino acids, or a monosaccharaide or oligosaccharide comprising 10 or fewer saccharide units, or a poly(*N*-carboxyalkyl)amine or a poly-oxy anion and the X^2 moiety is poly(*N*-carboxyalkyl)amine or a polyoxy-anion, or an amino acid or a peptide having an amino acid sequence of no more than 10 residues (including peptides wherein the carboxyl group of the carboxyl-terminal amino acid is reduced to an alcohol), or a monosaccharide or oligosaccharide comprising 10 or fewer saccharide units. In another preferred embodiment, B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

The invention also provides a cyclic peptide that is a somatostatin analogue as a

composition of matter comprising a somatostatin-receptor binding peptide having the formula:

Formula III

cyclo-A^4-$B^1B^2B^3B^4$-C^4-

where B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or a substituted derivative thereof; B^2 is D- or L-Trp or a substituted derivative thereof; B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or a substituted derivative thereof; B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib; C^4 is an L-amino acid; and A^4 is a lipophilic D-amino acid or a lipophilic L-(α-*N*-alkyl) amino acid or L-cysteine or L-proline or a substituted derivative thereof; . This moiety is a cyclic peptide moiety, where the amino terminus of the A^4 residue is covalently linked with the carboxyl terminus of the C^4 residue. In a preferred embodiment, B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine; and wherein the cyclic peptide is covalently linked to a radiolabel-binding moiety, wherein the radiolabel-binding moiety is not covalently linked to the moieties B^1, B^2, B^3, B^4 or A^4 of the peptide.

The invention also provides scintigraphic imaging agents comprising the cyclic peptide reagents of the invention wherein the radiolabel-binding moiety is stably complexed with a radioisotope. In one such embodiment is provided a scintigraphic imaging agent wherein the somatostatin analogue, cyclic peptide reagents of the invention are radiolabeled with technetium-99m. In other embodiments of the scintigraphic imaging agents of the invention the radioisotope is indium-111 or gallium-68. In still other embodiments, the scintigraphic imaging agents of the invention are cyclic peptides that are radiolabeled with iodine-123 or iodine-125.

The invention also provides radiotherapeutic agents that are the cyclic peptide reagents of the invention radiolabeled with a cytotoxic radioisotope that is selected from the group consisting of scandium-47, copper-67, gallium-72, yttrium-90, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, and bismuth-212. In preferred embodiments, the radioisotope is rhenium-186 or rhenium-188. In additional preferred embodiments, the cyclic peptides of the invention are radiolabeled with iodine-125, iodine-131 or astatine-211.

The invention further provides therapeutic agents comprising the cyclic peptide

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

reagents of the invention, optionally wherein the reagents form a complex with a non-radioactive metal, preferably rhenium. In this aspect of the invention, the cyclic peptide somatostatin analogues have increased *in vivo* stability compared with native somatostatin. Combination embodiments, wherein such a complex is also radiolabeled, either directly or *via* a radiolabel-binding moiety, are also provided by the invention and are within its scope. The somatostatin analogues of the invention are therapeutically useful in the alleviation of diseases or other ailments in humans or other animals.

The invention also provides pharmaceutical compositions comprising the somatostatin receptor-binding peptides of the invention in a pharmaceutically acceptable carrier.

The invention also provides a method for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the somatostatin analogues of the invention to the animal. In preferred embodiments, the amount of the somatostatin analogue administered is from about 0.1 to about 50 mg/kg body weight/day.

Another aspect of the present invention provides reagents for preparing scintigraphic imaging agents, each reagent comprising a peptide that is somatostatin analogue and is covalently linked to a radiolabel-binding moiety.

It is an advantage of the somatostatin analogues provided by this invention that the non-disulfide cyclic linkage contained therein is stable under the conditions of radiolabeling the covalently linked radiolabel-binding moiety. In contrast, for example, Tc-99m conjugation to a Tc-99m binding moiety covalently linked to native somatostatin, or to a somatostatin analogue having a disulfide bond, can result in reduction of the disulfide accompanied by a loss of biological activity. Such loss of biological activity can also occur *in vivo* using native somatostatin, or to any somatostatin analogue having a disulfide bond. The present invention is not subject to similar losses in biological activity *in vivo* because the non-disulfide cyclic linkages in each of the somatostatin analogues of the invention comprise stable covalent bonds.

It is another advantage of the somatostatin analogues provided by this invention that the cyclic covalent linkage acts to protect the peptide from degradation by exopepetidases. Further, the cyclic structure confers a degree of conformational rigidity to the peptide that

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

can act to enhance binding of the peptide to its biological target (i.e., the somatostatin receptor).

A first aspect of the reagents provided by the invention for preparing radiolabeled agents are reagents that are each comprised of a cyclic peptide that is a somatostatin analogue that is covalently linked to a radiolabel-binding moiety having the formula:

$$C(pgp)^{s}\text{-}(aa)\text{-}C(pgp)^{s}$$

where $(pgp)^{s}$ is hydrogen or a thiol protecting group and (aa) is an amino acid. In a preferred embodiment, the amino acid is glycine. In another preferred embodiment, the agent is a scintigraphic imaging agent. In yet another preferred embodiment, the agent is a radiotherapeutic agent.

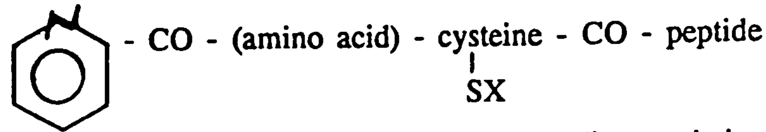
In a second embodiment, the invention provides cyclic peptide reagents capable of being radiolabeled to form radiodiagnostic and radiotherapeutic agents, each comprising a somatostatin analogue that is covalently linked to a radiolabel-binding moiety of of formula:

$$A\text{-}CZ(B)\text{-}[C(R'R'')]_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC or R''''; B is H, SH or -NHR''', -N(R''')-(peptide) or R''''; Z is H or R''''; X is SH or -NHR''', -N(R''')-(peptide) or R''''; R', R'', R''' and R'''' are independently H or straight or branched chain or cyclic lower alkyl; n is 0, 1 or 2; and: (1) where B is -NHR''' or -N(R''')-(peptide), X is SH and n is 1 or 2; (2) where X is -NHR''' or -N(R''')-(peptide), B is SH and n is 1 or 2; (3) where B is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, X is SH and n is 0 or 1; (4) where A is H or R'''', then where B is SH, X is -NHR''' or -N(R''')-(peptide) and where X is SH, B is -NHR''' or -N(R''')-(peptide); (5) where X is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH; (6) where Z is methyl, X is methyl, A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH and n is 0; and (7) where Z is SH and X is SH, n is not 0; and wherein the thiol moiety is in the reduced form. In a preferred embodiment, the agent is a scintigraphic imaging agent. In yet another preferred embodiment, the agent is a radiotherapeutic agent.

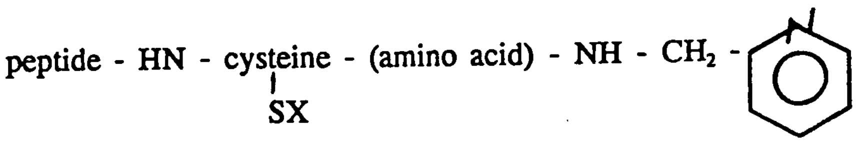
In another embodiment, the invention provides cyclic peptide reagents capable of being radiolabeled with a radioisotope to form radiodiagnostic and radiotherapeutic agents, each comprising a somatostatin analogue that is covalently linked to a radiolabel-binding moiety

of formula:



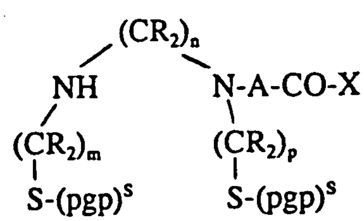
[for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolinic acid (Pic)-based moieties]

or

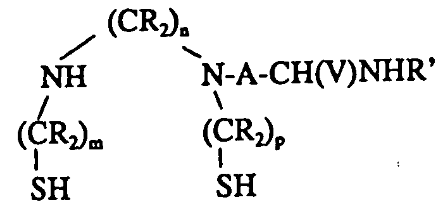


wherein X is H or a protecting group; (amino acid) is any amino acid and the radiolabel-binding moiety is covalently linked to the peptide. For purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolylamine (Pica)-based moieties. In a preferred embodiment, the amino acid is glycine and X is an acetamidomethyl protecting group. In another preferred embodiment, the agent is a scintigraphic imaging agent. In yet another preferred embodiment, the agent is a radiotherapeutic agent.

Yet another embodiment of the invention provides cyclic peptide reagents capable of being radiolabeled with a radioisotope for imaging sites within a mammalian body or for radiotherpeutic purposes, each comprising a somatostatin analogue that is covalently linked to a radiolabel-binding moiety that is a bisamino-bisthiol radiolabel-binding moiety. The bisamino bisthiol radiolabel-binding moiety in this embodiment of the invention has the formula:



wherein each R can be independently H, CH_3 or C_2H_5; each $(pgp)^S$ can be independently a thiol protecting group or H; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; and X is peptide; or



wherein each R is independently H, CH_3 or C_2H_5; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; V is H or CO-peptide; R' is H or peptide; provided that when V is H, R' is peptide and when R' is H, V is peptide. For purposes of this invention, radiolabel-binding moieties having these structures will be referred to as "BAT" moieties. In a preferred embodiment, the agent is a scintigraphic imaging agent. In yet another preferred embodiment, the agent is a radiotherapeutic agent.

This invention provides methods for preparing peptide reagents of the invention by chemical synthesis *in vitro*. In a preferred embodiment, cyclic peptides are synthesized by solid phase peptide synthesis.

This invention provides reagents for preparing a radiolabled somatostatin receptor-binding agent comprising the somatostatin receptor-binding cyclic peptides of the invention covalently linked to a radiolabel-binding moiety. In a preferred embodiment, the reagent is radioactively labeled with Tc-99m. In another preferred embodiment, the reagent is radioactively labeled with ^{186}Re or ^{188}Re.

The invention also comprises agents that are complexes of the cyclic peptide reagents of the invention with a radioisotope, as well as methods for radiolabeling the peptide reagents of the invention. For example, scintigraphic imaging agents provided by the invention comprise Tc-99m labeled complexes formed by reacting the peptide reagents of the invention with Tc-99m in the presence of a reducing agent. Preferred reducing agents include but are not limited to dithionite ion, stannous ion and ferrous ion. Such Tc-99m complexes of the invention are also formed by labeling the peptide reagents of the invention with Tc-99m by ligand exchange of a prereduced Tc-99m complex as provided herein.

The invention also provides kits for preparing radiolabeled somatostatin analogue cyclic peptides from the peptide reagents of the invention. Kits for radiolabeling the peptide reagents of the invention are comprised of a sealed vial containing a predetermined quantity

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

of a peptide reagent of the invention and a sufficient amount of reducing agent to radiolabel the peptide. In a preferred embodiment, the radiolabeled somatostain analogue is a scintigraphic imaging agent. Also preferred is radiolabeling the peptide reagents of the invention with Tc-99m. Kits for preparing radiotheapeutic agents are also provided, wherein the preferred radioisotopes are rhenium-186 and rhenium-188.

This invention provides methods for using the radiolabeled peptide reagents of the invention diagniostically and therapeutically. In one embodiment of the invention, methods are provided for using scintigraphic imaging agents that are Tc-99m labeled peptide reagents for imaging sites within a mammalian body by obtaining *in vivo* gamma scintigraphic images. These methods comprise administering an effective diagnostic amount of radiolabeled peptide reagents of the invention and detecting the gamma radiation emitted by the radiolabel localized at the site within the mammalian body.

The invention also provides methods for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the radiolabeled somatostatin-binding peptide reagents of the invention to the animal. In preferred embodiments, the reagent is radioactively labeled with ^{186}Re or ^{188}Re.

The cyclic peptides and cyclic peptide reagents of the invention may also be comprised of a polyvalent linking moiety. Polyvalent linking moieties of the invention are comprised of at least 2 identical linker functional groups capable of covalently bonding to somatostatin analogue cyclic peptides or radiolabel-binding moieties. Preferred linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups. In preferred embodiments, the polyvalent linking moieties are comprised of *bis*-succinimidylmethylether (BSME), 4-(2,2-dimethylacetyl)benzoic acid (DMBA), *N*-[2-(*N'*,*N'*-*bis*(2-succinimido-ethyl)aminoethyl)]-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide (BAT-BS), *tris*(succinimidylethyl)amine (TSEA), *bis*-succinimidohexane (BSH), 4-(O-CH_2CO-Gly-Gly-Cys.amide)-2-methylpropiophenone (ETAC) or a derivative thereof.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides cyclic peptides that are somatostatin analogues and that are not comprised of a disulfide bond. Such somatostatin analogues thereby possess increased *in vivo* stability compared with native somatostatin. These cyclic peptides are themselves therapeutic agents for alleviating diseases and other ailments in animals including humans.

Also provided by the invention are cyclic peptides that may be radioiodinated or radioastatinated and which are thereby useful in radiotherapeutic and radiodiagnostic applications.

Another embodiment of these cyclic peptides that is provided by this invention are cyclic peptide reagents wherein the cyclic peptides of the invention are covalently linked to a radiolabel-binding moiety. Such cyclic peptide reagents are capable of being radiolabeled to provide radiodiagnostic or radiotherapeutic agents. One example of a radiodiagnostic application using the radiolabeled agents of the invention is scintigraphic imaging, wherein the location and extent of somatostatin receptor-bearing tumors may be determined. The cyclic peptide reagents of the invention can also advantageously be radiolabeled with cytotoxic radioisotopes such as rhenium-186 or rhenium-188 for radiotherapeutic uses. The cyclic peptide reagents of the invention are also useful in preparing complexes with non-radioactive metals, said complexes being useful therapeutically.

The invention provides a method for using the somatostatin analogues of the invention to alleviate diseases or other ailments in animals, preferably humans. These diseases and ailments include but are not limited to diabetes and diabetes-related retinopathy, cirrhosis of the liver and hepatitis infection, bleeding ulcers and other gastrointestinal bleeding, pancreatitis, central nervous system disorders, endocrine disorders, Alzheimer's disease, acromegaly and other diseases and disorders related to the production of inappropriate levels of growth hormone *in vivo*, and cancer, particularly those cancers whose growth is dependent or influenced by growth hormone production. Dosages of the somatostatin analogues provided by the invention may be the same as those dosages of native somatostatin routinely used for treatment of the above or other diseases, or less of the compounds of the invention may be administered due to their longer *in vivo* half-life.

Labeling with Tc-99m is an advantage of the present invention because the nuclear

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator. Other radionuclides may also be used in the practice of the invention as disclosed herein.

The term scintigraphic imaging agent as used herein is meant to encompass a radiolabeled agent capable of being detected with a radioactivity detecting means (including but not limited to a gamma-camera, a Geiger-Muller counter and a scintillation detector probe).

Radiotherapeutic embodiments of the invention, on the other hand, are advantageously labeled with a cytotoxic radioisotope, including but not limited to scandium-47, copper-67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, astatine-211 and bismuth-212, most preferably ^{186}Re or ^{188}Re. Such embodiments are useful in the treatment of somatostatin-related diseases or other ailments in animals, preferably humans, including but not limited to cancer and other diseases characterized by the growth of malignant or benign tumors capable of binding somatostatin or somatostatin analogues *via* the expression of somatostatin receptors on the cell surface of cells comprising such tumors.

In the radiolabel-binding moieties and cyclic peptides covalently linked to such moieties that contain a thiol covalently linked to a thiol protecting groups [(pgp)^S] provided by the invention, the thiol-protecting groups may be the same or different and may be but are not limited to:

-CH_2-aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);

-CH-$(aryl)_2$, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);

-C-$(aryl)_3$, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);

-CH_2-(4-methoxyphenyl);

-CH-(4-pyridyl)$(phenyl)_2$;

-$C(CH_3)_3$

-9-phenylfluorenyl;

-CH_2NHCOR (R is unsubstituted or substituted alkyl or aryl);

-CH_2-NHCOOR (R is unsubstituted or substituted alkyl or aryl);

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

-CONHR (R is unsubstituted or substituted alkyl or aryl);

-CH_2-S-CH_2-phenyl

Preferred protecting groups have the formula -CH_2-NHCOR wherein R is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxycarbonyl. The most preferred protecting group is an acetamidomethyl group.

Each somatostatin receptor-binding cyclic peptide-containing embodiment of the invention is comprised of a sequence of amino acids. The term amino acid as used in this invention is intended to include all L- and D- amino acids, naturally occurring and otherwise. Reagents comprising somatostatin receptor-binding peptides provided by the invention include but are not limited to the following illustrative examples of the peptide embodiments of the invention:

Structures Depicted by Single-Letter Code

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy

CH_2CO.FYW$_D$KTFC.amide

CH_2CO.FFW$_D$KTF.Hhc.amide

cyclo.CYW$_D$KVC

CH_2CO.FFW$_D$KTFC.amide

cyclo.(*N*-CH_3)F.YW$_D$KV.K.(BAT)

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.K(ϵ-K)GC.amide)

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.C$_{Acm}$GC$_{Acm}$.amide)

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.CGC.amide)

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.CGC)

CH_2CO.FFW$_D$KTFC.[BAM]

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.(ϵ-K)GC.amide)

cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.GGC.amide)

cyclo.(*N*-CH_3)F.YW$_D$KV.E.(BAM)

2CH_2CO.NFFW$_D$KTFTC

CH_2CO.FFW$_D$KTFC

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}$

$\underline{CH_2CO.FFW_DKTFC}(\varepsilon\text{-}K)GC$.amide

$\underline{CH_2CO.FFW_DKTFC}C_{Acm}GC_{Acm}$.amide

$\underline{CH_2CO.FFW_DKTF.Hcy}$

$\underline{CH_2CO.YW_DKTC}$

$\underline{CH_2CO.YW_DKT.Hcy}$.amide

$\underline{CH_2CO.YW_DKT.Hhc}.T(CH_2OH)$

$\underline{CH_2CO.YW_DKTC}TGGC_{Mob}$.amide

$\underline{CH_2CO.YW_DKT.Hhc}$

D-*PHENYL*-$CH_2\underline{CH_2CO.YW_DKTC}$

$\underline{CH_2CO.FW_DKT.Pen}$

$\underline{CH_2CO.FW_DKTHcy}$.amide

$\underline{CH_2CO.YW_DKTCT}$

$\underline{CH_2CO.YW_DKTC}T(CH_2OH)$

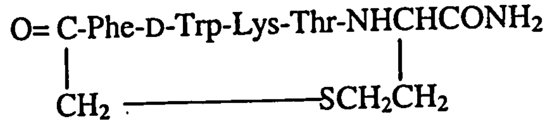
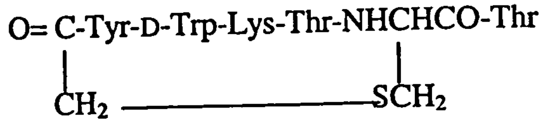
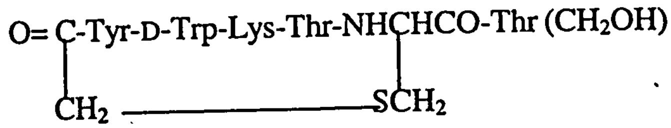
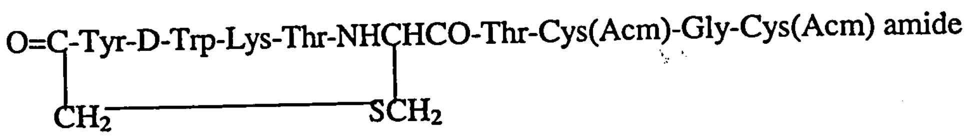
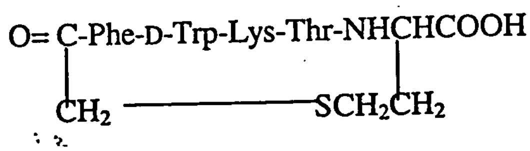
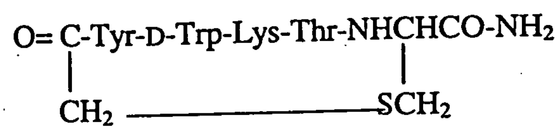
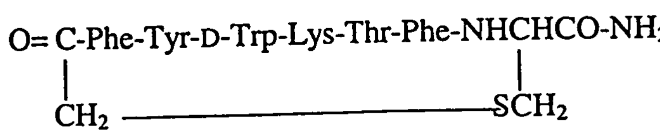
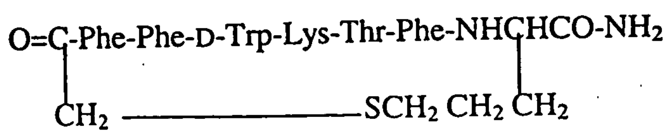
$\underline{CH_2CO.YW_DKTC}TC_{Acm}GC_{Acm}$.amide

$\underline{CH_2CO.FW_DKTHcy}$

$\underline{CH_2CO.YW_DKTC}$.amide

where the above structures, represented by single-letter amino acid sequence code correspond to the following three-letter amino acid sequence code:

Structures Depicted by Single-Letter Code	Structures Depicted by Three-Letter Code
$\underline{CH_2CO.YW_DKT.Hhc}$	O= C-Tyr-D-Trp-Lys-Thr-NHCHCOOH CH_2 —— $SCH_2CH_2CH_2$
D-PHENYL-$CH_2\underline{CH_2CO.YW_DKTC}$	D-$PhCH_2CHCO$-Tyr- D-Trp-Lys-Thr-NHCHCOOH CH_2 —— SCH_2
$\underline{CH_2CO.FW_DKT.Pen}$	O= C-Phe-D-Trp-Lys-Thr-NHCHCOOH CH_2 —— $SC(CH_3)_2$

Structures Depicted by Single-Letter Code	Structures Depicted by Three-Letter Code
CH_2CO.FW$_D$KTHcy.amide	
CH_2CO.YW$_D$KTCT	
CH_2CO.YW$_D$KTCT(CH_2OH)	
CH_2CO.YW$_D$KTCTC$_{Acm}$GC$_{Acm}$.amide	
CH_2CO.FW$_D$KTHcy	
CH_2CO.YW$_D$KTC.amide	
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy	*cyclo*.(*N*-CH_3)Phe-Tyr-D-Trp-Lys-Val-Hcy
CH_2CO.FYW$_D$KTFC.amide	
CH_2CO.FFW$_D$KTF.Hhc.amide	
cyclo.CYW$_D$KVC	cyclo Cys-Tyr- D-Trp-Lys-Val-Cys (cyclic peptide, not disulfide)

Structures Depicted by Single-Letter Code	Structures Depicted by Three-Letter Code
$\underline{CH_2CO.FFW_DKTFC}$.amide	O=C-Phe-Phe-D-Trp-Lys-Thr-Phe-NHCHCO-NH_2 CH_2 —————————— SCH_2
cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.K.(BAT)}$	*cyclo*.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Lys(BAT)}}$

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.K(\varepsilon\text{-}K)GC.\text{amide})$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{-Lys}(\varepsilon\text{-Lys})\text{-Gly-Cys-amide})$

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.C_{Acm}GC_{Acm}.\text{amide})$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{-Cys(Acm)-Gly-Cys(Acm)-amide})$

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.CGC.\text{amide})$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{-Cys-Gly-Cys-amide})$

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.CGC)$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{-Cys-Gly-Cys})$

$\underline{CH_2CO.FFW_DKTFC}$.(BAM)	O=C-Phe-Phe-D-Trp-Lys-Thr-Phe-NHCHCO (BAM) CH_2 —————————— SCH_2

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.(\varepsilon\text{-}K)GC.\text{amide})$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{-}(\varepsilon\text{-Lys})\text{-Gly-Cys-amide})$

cyclo.$\underline{(N\text{-}CH_3)F.YW_DKV.Hcy}(CH_2CO.GGC.\text{amide})$

cyclo.$\underline{(N\text{-}CH_3)\text{Phe-Tyr-D-Trp-Lys-Val-Hcy}}(CH_2CO\text{- Gly -Gly-Cys-amide})$

cyclo.(*N*-CH_3)F.YW_DKV.E.(BAM) *cyclo*.(*N*-CH_3)Phe-Tyr-D-Trp-Lys-Val-Glu(BAM)

Structures Depicted by Single-Letter Code	Structures Depicted by Three-Letter Code
CH_2CO.NFFW_DKTFTC	O= C-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NHCHCOOH CH_2 ——— SCH_2
CH_2CO.FFW_DKTFC	O= C- Phe-Phe-D-Trp-Lys-Thr-Phe-NH CHCOOH CH_2 ——— SCH_2

CH_2CO.FFW_DKTFC(ε-K)GC.amide

O=C-Phe-Phe-D-Trp-Lys-Thr-Phe-NHCHCO-(ε-Lys)-Gly-Cys-amide
CH_2 ——— SCH_2

CH_2CO.FFW_DKTFCC_{Acm}GC_{Acm}.amide

O=C-Phe-Phe-D-Trp-Lys-Thr-Phe-NHCHCO-Cys(Acm)-Gly-Cys(Acm)-amide
CH_2 ——— SCH_2

CH_2CO.FFW_DKTF.Hcy	O= C- Phe-Phe-D-Trp-Lys-Thr-Phe-NH CHCOOH CH_2 ——— SCH_2CH_2
CH_2CO.YW_DKTC	O= C-Tyr-D-Trp-Lys-Thr-NHCHCOOH CH_2 ——— SCH_2
CH_2CO.YW_DKT.Hcy.amide	O= C-Tyr-D-Trp-Lys-Thr-NHCHCOOH CH_2 ——— SCH_2CH_2
CH_2CO.YW_DKT.Hhc.T(CH_2OH)	O= C-Tyr-D-Trp-Lys-Thr-NHCHCO-Thr(CH_2OH) CH_2 ——— $SCH_2CH_2CH_2$

CH_2CO.YW_DKTCTGGC_{Mob}.amide

As used herein, the following amino acids and amino acid analogues are intended to be represented by the following abbreviations: Ac is an acetyl group; ma is mercaptoacetic acid group; Aca is 6-aminocaproic acid; Hcy is homocysteine; Hhc is homohomocysteine, which is (3-mercaptopropyl)glycine; Pen is penicillamine; Mob is the sulfhydrul protecting group 4-methoxybenzyl; Acm is the sulfhydryl protecting group acetamidomethyl; Aib is aminoisobutyric acid; Nal is 2-naphthylalanine; Ain is 2-aminoindan-2-carboxylic acid; Hly is homolysine; Achxa is 4-amino-cyclohexylalanine; Amf is 4-aminomethylphenylalanine; Aec is S-(2-aminoethyl)cysteine; Apc is S-(3-aminopropyl)cysteine; Aes is O-(2-aminoethyl)serine; Aps is O-(3-aminopropyl)serine; Abu is 2-aminobutyric acid; Nva is norvaline; Aca is 6-aminocaproic acid; F_D is D-phenylalanine; W_D is D-tryptophan; Y_D is D-tyrosine; Cpa is L-(4-chlorophenyl)alanine; Thp is 4-amino-tetrahydrothiopyran-4-carboxylic acid; D-Nal is D-2-naphthylalanine; Dpg is dipropylglycine; and Nle is norleucine. All naturally-occurring amino acids are abbreviated using standard abbreviations (which can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33. for the purposes of this invention, the naturally-occuring amino acids are characterized as <u>lipophilic</u> (alanine, isoleucine, leucine, methionine, phenylalanine, tyrosine, proline, tryptophan and valine, as well as *S*-alkylated derivatives of cysteine), <u>hydrophilic</u> (asparagine, glutamine, threonine, serine), <u>acidic</u> (glutamic acid and aspartic acid), <u>basic</u> (arginine, histidine and lysine). $T(CH_2OH)$ represents a threoninol residue, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, incorporated into the peptide using the procedure of Neugebauer *et al.* (1990, <u>Peptides: Proceedings of the 11th American Peptide Symposium</u>, pp. 1020-21). ε-K is intended to represent a covalent linkage *via* the ε-amino group on the sidechain of a lysine residue. Pic is picolinoyl (pyridine-2-carbonyl); Pica is picolylamine (2-(aminomethyl)pyridine); [BAT] represents N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; K.(BAT) and Lys.(BAT) represent the amino acid lysine, acylated at the ε-amino group on the amino acid sidechain to [BAT]; [BAM] is (N^1,N^4-*bis*(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; E.(BAM) and Glu.(BAM) represent the amino acid glutamic acid having a γ-amide linkage between the sidechain carboxylic acid group of glutamic acid and a [BAM]-derived primary amino group; [BAT-BM] is *N*-[2-(*N'*,*N'*-*bis*(2-

maleimidoethyl)aminoethyl]-N^9-(t-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; [BAT-BS] is *N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl]-N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanamide; [BMME] is *bis*-maleimidomethylether; [BSME] is *bis*-succinimidomethylether; and [DTPA] is diethylenetriaminepentaacetic acid. Hcy(alkyl group) is homocyteine, *S*-alkylated with the group in parenthesis.

The convention used herein of representing by underlining a covalent bond between atoms and groups of atoms, such as the amino terminus and carboxyl terminus resulting in the cyclic peptides of the invention, or similar representations of covalent bonding between the sidechain sulfur atom of a cysteine residue or derivative thereof and an amino terminal acyl group or other residue will also be understood by those with skill in the art. The use of the term "*cyclo*" herein is intended to indicate that the peptide is cyclized by formation of a covalent bond between the atoms of the amino terminal substituted or unsubstituted amino group and the carboxyl terminus of the peptide.

For the purposes of this invention the term "poly(*N*-carboxyalkyl)amine" in intended to describe a series of compounds exemplified by nitrilotriacetic acid, iminodiacetic acid, ethylenediaminetetraacetic acid (EDTA) and diethylenepentaacetic acid (DTPA).

For the purposes of this invention the term "polyoxyanion" is intended to encompass sulfates, phosdphates, sulfonates, phosphonates like compounds.

Somatostatin analogue peptides of the present invention can be chemically synthesized *in vitro*. Peptides of the present invention can generally advantageously be prepared on a peptide synthesizer. The peptides of this invention can be synthesized wherein the radiolabel-binding moiety is covalently linked to the peptide during chemical synthesis *in vitro*, using techniques well known to those with skill in the art. Such peptides covalently-linked to the radiolabel-binding moiety during synthesis are advantageous because specific sites of covalent linkage can be determined.

Radiolabel binding moieties of the invention may be introduced into the target somatostatin analogue peptides during peptide synthesis. For embodiments comprising picolinic acid [(Pic-); *e.g.*, Pic-Gly-Cys(protecting group)-], the radiolabel-binding moiety can

be synthesized as the last (i.e., amino-terminal) residue in the synthesis. In addition, the picolinic acid-containing radiolabel-binding moiety may be covalently linked to the ε-amino group of lysine to give, for example, αN(Fmoc)-Lys-εN[Pic-Gly-Cys(protecting group)], which may be incorporated at any appropriate position in the peptide chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target somatostatin analogue peptide.

Similarly, the picolylamine (Pica)-containing radiolabel-binding moiety [-Cys(protecting group)-Gly-Pica] can be prepared during peptide synthesis by including the sequence [-Cys(protecting group)-Gly-] at the carboxyl terminus of the peptide chain. Following cleavage of the peptide from the resin the carboxyl terminus of the peptide is activated and coupled to picolylamine. This synthetic route requires that reactive side-chain functionalities remain masked (protected) and do not react during the conjugation of the picolylamine.

This invention also provides small synthetic peptides that are somatostatin analogues and incorporate bisamine bisthiol (BAT) chelators that may be labeled with Tc-99m.

This invention provides for the incorporation of these chelators into virtually any position in the peptide, *via* covalently linkage to any appropriate functional group of the peptide, except that the chelating moieties of the invention are not covalently linked to functional groups comprising the amino acid side chains of the amino acids B^1, B^2, B^3 or B^4.

In forming a complex of radioactive technetium with the reagents of this invention, the technetium complex, preferably a salt of Tc-99m pertechnetate, is reacted with the reagent in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. Means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a reagent of the invention to be labeled and a sufficient amount of reducing agent to label the reagent with Tc-99m. Alternatively, the complex may be formed by reacting a reagent of this invention with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex may be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the Tc-99m pertechnetate salts useful with the present invention are included the

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

In a preferred embodiment of the invention, a kit for preparing technetium-labeled peptides is provided. An appropriate amount of the peptide reagent is introduced into a vial containing a reducing agent, such as stannous chloride, in an amount sufficient to label the peptide with Tc-99m. An appropriate amount of a transfer ligand as described (such as tartrate, citrate, gluconate or mannitol, for example) can also be included. The kit may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The components of the kit may be in liquid, frozen or dry form. In a preferred embodiment, kit components are provided in lyophilized form.

Tc-99m labeled imaging reagents according to the present invention may be prepared by the addition of an appropriate amount of Tc-99m or Tc-99m complex into the vials and reaction under conditions described in Example 2 hereinbelow.

Radioactively-labeled scintigraphic imaging agents provided by the present invention are provided having a suitable amount of radioactivity. In forming Tc-99m radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 millicurie (mCi) to 100 mCi per mL.

The imaging reagents provided by the present invention can be used for visualizing organs such as the kidney for diagnosing disorders in these organs, and tumors, in particular gastrointestinal tumors, myelomas, small cell lung carcinoma and other APUDomas, endocrine tumors such as medullary thyroid carcinomas and pituitary tumors, brain tumors such as meningiomas and astrocytomas, and tumors of the prostate, breast, colon, and ovaries can also be imaged. In accordance with this invention, the Tc-99m labeled peptide reagents are administered in a single unit injectable dose. The Tc-99m labeled peptide reagents provided by the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours

or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

The somatostatin receptor-binding cyclic peptides and non-radioactive metal complexes of the cyclic peptide reagents of the invention may be used clinically as therapeutic agents to promote regression of certain types of tumors, particularly those that express somatostatin receptors. The somatostatin analogue cyclic peptides of the invention can also be used to reduce the hormonal hypersecretion that often accompanies certain cancers, such as the APUDomas. Peptides of the invention used as therapeutic agents may be administered by any appropriate route, including intravenous, intramuscular or by mouth, and in any acceptable pharmaceutical carrier, in doses ranging from about 0.1 to about 49 mg/kgbody weight/day.

This invention also provides peptides radiolabled with cytotoxic radioisotopes such as rhenium-186 or rhenium-188 that may be used for radiotherapy of certain tumors as described above. For this purpose, an amount of radioactive isotope from about 10mCi to about 200mCi may be administered *via* any suitable clinical route, preferably by intravenous injection.

The methods for making and labeling these compounds are more fully illustrated in the following Examples. These Examples illustrate certain aspects of the above-described method and advantageous results, and are shown by way of illustration and not limitation.

EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzotriazol-1-yl)-1,1,3,3-

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

tetramethyluronium hexafluorophosphate/ hydroxybenzotriazole (HBTU/HOBT), and using *p*-hydroxymethylphenoxy-methylpolystyrene (HMP) resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Where appropriate, the following amino acid derivatives were synthesized. Homocysteine was prepared by alkaline hydrolysis of L-homocysteine lactone. Threoninol residues, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, can be introduced into the peptides of the invention where appropriate using the procedure of Neugebauer *et al.* (1990, Peptides: Proceedings of the 11th American Peptide Symposium, pp. 1020-21). Fmoc.Hcy(Trt) and Fmoc.Pen(Trt) were prepared from the appropriate amino acids by tritylation with triphenylmethanol in TFA, followed by Fmoc derivitization as described by Atherton *et al.* (1989, Solid Phase Peptide Synthesis, IRL Press: Oxford). Fmoc.homohomocysteine(Trt) was prepared by reducing *N,N-bis*-Boc-glutamic acid-α-methyl ester with borane-THF, followed by mesylation and reaction with trityl-mercaptide, followed by removal of the Boc groups with BF_3OEt_2 in acetic acid, and then Fmoc derivitization as described above. *phenyl*-$CH_2CHBrCOOH$ was prepared by treating phenylalanine (in a solution of water and TFA/ saturated with NaBr) with sodium nitrite, followed by distillation to recover the pure product.

Where appropriate, 2-chloroacetyl, 2-bromoacetyl and 2-bromo-3-phenylpropionyl groups were introduced either by using the appropriate 2-halo acid as the last residue coupled during SPPS, or by treating the N-terminus free amino acid peptide bound to the resin with either 2-halo acid/ diisopropylcarbodiimide/*N*-hydroxysuccinimide/NMP or 2-halo acid anhydride/ diisopropylethylamine/NMP.

Where appropriate, HPLC-purified 2-haloacylated peptides were cyclized by stirring an 0.1-1.0 mg/mL solution in phosphate or bicarbonate buffer or dilute ammonium hydroxide (pH 8.0), optionally containing 0.5-1.0 mM EDTA, or acetonitrile or THF for 1-48 h followed optionally by acidification with acetic acid, lyophilization and HPLC purification.

Where appropriate, [BAM] (N^1,N^4-*bis*(2-mercapto-2-methylpropyl)-1,4,10-triazadecane) was conjugated to the peptide by first activating the peptide carboxylate with a mixture of diisopropylcarbodiimide/ N-hydroxysuccinimide or HBTU/HOBt in DMF, NMP or methylene

chloride, followed by coupling in the presence of diisopropylethylamine. After coupling, the conjugates were deprotected as described above.

Where appropriate, BSME adducts were prepared by reacting single thiol-containing peptides (5 to 50 mg/mL in DMF buffered to pH 7 with N-methylmorpholine or N-ethylmorpholine, or 50mM sodium phosphate buffer, pH 7-8, optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BMME (*bis*-maleimidomethylether) pre-dissolved in acetonitrile at room temperature for approximately 1-18 hours. The solution was concentrated and the product was purified by HPLC.

Where appropriate, TSEA adducts were prepared by reacting single thiol-containing peptide (at concentrations of 10 to 100 mg/mL peptide in DMF buffered to pH 7 with N-methylmorpholine or N-ethylmorpholine, or 5 to 50 mg/mL peptide in 50mM sodium phosphate, pH 7-8, optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.33 molar equivalents of TMEA (*tris*(2-maleimidoethyl)amine) pre-dissolved in acetonitrile or DMF, with or without 1 molar equivalent of triethanolamine, at room temperature for approximately 1-18h. Such reaction mixtures containing adducts were concentrated and the adducts were then purified using HPLC.

Where appropriate, BAT-BS (*N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl)]-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide) adducts were prepared by reacting single thiol-containing peptide (at concentrations of 2 to 50 mg/mL peptide in DMF buffered to pH 7 with N-methylmorpholine or N-ethylmorpholine, or in 50mM sodium phosphate (pH 7-8), optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BAT-BM (*N*-[2-(*N'*,*N'*-*bis*(2-maleimidoethyl)aminoethyl)]-N^9-(*t*-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide) pre-dissolved in acetonitrile or THF, at room temperature for approximately 1-18h. The solution was then evaporated to dryness and [BAT-BS]-peptide conjugates deprotected by treatment with 10mL TFA and 0.2mL triethylsilane for 1h. The solution was concentrated, the product adducts precipitated with ether, and then purified by HPLC.

Where appropriate, the [DTPA] moiety can be introduced using the method of Bakker *et al.* (1991, Life Sci. 49: 1583-1591, hereby incorporated by reference).

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

Where appropriate, peptide precursors were cyclized (between the amino- and carboxyl-termini) by reaction of the sidechain-protected, N-terminal free amine and C-terminal free acid with diphenylphosphorylazide.

Resin-bound products were routinely cleaved using a solution of trifluoroacetic acid or trifluoroacetic acid and methylene chloride, optionally containing water, thioanisole, ethanedithiol, and triethylsilane, prepared in ratios of 100 : 5 : 5 : 2.5 : 2 for 0.5 - 3 h at room temperature. Crude peptides were purified by preparative high pressure liquid chromatography (HPLC) using a Waters Delta Pak C18 column and gradient elution using 0.1% trifluoroacetic acid (TFA) in water modified with acetonitrile. Acetonitrile was evaporated from the eluted fractions which were then lyophilized. The identity of each product was confirmed by fast atom bombardment mass spectroscopy (FABMS) or by electrospray mass spectroscopy (ESMS).

Somatostatin analogues synthesized as provided herein, as well as the products of such synthesis identified by FABMS, are shown in Table I below.

EXAMPLE 2

A General Method for Radiolabeling with Tc-99m

0.1 mg of a peptide prepared as in Example 1 was dissolved in 0.1 mL of water or 50/50 ethanol/water or phosphate-buffered saline or 50 mM potassium phosphate buffer (pH = 5, 6 or 7.4). Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc.) with 1.0 mL of Tc-99m sodium pertechnetate containing up to 200 mCi and allowed to stand for 15 minutes at room temperature. 25 μl of Tc-99m gluceptate was then added to the peptide and the reaction allowed to proceed at room temperature or at 100°C for about 15-30 min and then filtered through a 0.2 μm filter.

The Tc-99m labeled peptide purity was determined by HPLC using the following conditions: a Waters Delta Pak RP-18, 5μ, 4.6mm x 220mm analytical column was loaded with each radiolabeled peptide, and the peptides eluted at a solvent flow rate equal to 1 mL/min. Gradient elution was performed beginning with 100% solvent A (0.1% CF_3COOH/H_2O) and ending with 100% solvent B_{90} (0.1% CF_3COOH/90% CH_3CN/H_2O) over the course of 10-20 min.

Radioactive components were detected using an in-line radiometric detector linked to an integrating recorder. Tc-99m gluceptate and Tc-99m sodium pertechnetate elute between 1 and 4 minutes under these conditions, whereas the Tc-99m labeled peptides eluted after a much greater amount of time, as illustrated in Table I below.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

TABLE I

Peptide	MH+ FABMS	RCY (%)	R_t (min)
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy	855	-	-
CH$_2$CO.FYW$_D$KTFC.amide	1033	-	-
CH$_2$CO.FFW$_D$KTF.Hhc.amide	1046	-	-
cyclo.CYW$_D$KVC	783	98[1]	11.4[1]
CH$_2$CO.FFW$_D$KTFC.amide	1017	-	-
cyclo.(*N*-CH$_3$)F.YW$_D$KV.K(BAT)	1185	90[1]	13.3, 14.4[1]
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.K(ϵ-K)GC.amide)	1328	nd	nd
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.C$_{Acm}$GC$_{Acm}$.amide)	1318	nd	nd
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.CGC.amide)	1176	99[4]	16.1[3]
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.CGC)	1177	99[4]	15.8, 17.8[3]
CH$_2$CO.FFW$_D$KTFC[BAM]	1322	99	18.8
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.(ϵ-K)GC.amide)	1201	99[3]	15.3[3]
cyclo.(*N*-CH$_3$)F.YW$_D$KV.Hcy(CH$_2$CO.GGC.amide)	1129	98[4]	15.1, 17.2[3]
cyclo.(*N*-CH$_3$)F.YW$_D$KV.E(BAM)	1171	98[1]	12.3, 13.6[1]
CH$_2$CO.NFFW$_D$KTFTC	1234	-	-
CH$_2$CO.FFW$_D$KTFC	1018	-	-
CH$_2$CO.FFW$_D$KTFC(ϵ-K)GC.amide	1305	99[4]	16.5[3]
CH$_2$CO.FFW$_D$KTFCC$_{Acm}$GC$_{Acm}$.amide	1422	99	15.1-16.9
CH$_2$CO.FFW$_D$KTF.Hcy	1032	-	-
CH$_2$CO.YW$_D$KTC	740	-	-
CH$_2$CO.YW$_D$KT.Hcy.amide	768	-	-
CH$_2$CO.YW$_D$KT.Hhc.T(CH$_2$OH)	855	-	-
CH$_2$CO.YW$_D$KTCTGGC$_{Mob}$.amide	1178	-	-
CH$_2$CO.YW$_D$KT.Hhc	769	-	-
D-*phenyl*-CH$_2$CHCO.YW$_D$KTC	830	-	-
CH$_2$CO.FW$_D$KT.Pen	752	-	-
CH$_2$CO.FW$_D$KTHcy.amide	737	-	-
CH$_2$CO.YW$_D$KTCT	841	-	-
CH$_2$CO.YW$_D$KTCT(CH$_2$OH)	828	-	-
CH$_2$CO.YW$_D$KTCTC$_{Acm}$GC$_{Acm}$.amide	1246	94[2]	16.6, 16.9[2]
CH$_2$CO.FW$_D$KTHcy	738	-	-
CH$_2$CO.YW$_D$KTC.amide	740	-	-

* The following labeling conditions were used with the appropriate peptides:

1. The peptide is dissolved in water and labeled at room temperature.
2. The peptide is dissolved in water and labeled at 100°C (15 min).
3. The peptide is dissolved in 10% hydroxypropylcyclodextrin and labeled at room temperature.
4. The peptide is dissolved in 50% ethanol/water and labeled at room temperature.

** HPLC methods:

general: solvent A = 0.1% CF3COOH/H_2O
solvent B_{90} = 0.1% CF_3COOH/90% CH_3CN/H_2O
solvent flow rate = 1 mL/min

Vydak column = Vydak 218TP54 RP-18, 5μ x 220mm x 4.6mm analytical column with guard column
Waters column = Waters Delta-Pak C18, 5μm, 39 X 150mm

Method 1:	Waters column	100% A to 100% B_{90} in 10 min
Method 2:	Vydak column	100% A to 100% B_{90} in 10 min
Method 3:	Waters column	100% A to 100% B_{90} in 20 min

Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33; Ac = acetyl; Acm = acetamidomethyl; ma = mercaptoacetic acid; Mob = 4-methoxybenzyl; Aca = 6-aminocaproic acid; Hly = homolysine; Apc = L-[S-(3-aminopropyl)cysteine; F_D = D-phenylalanine; W_D= D-tryptophan; $2Y_D$ = D-tyrosine; Cpa = L-(4-chlorophenyl)alanine; Thp = 4-amino-tetrahydrothiopyran-4-carboxylic acid; D-Nal = D-2-naphthylalanine; Dpg = dipropylglycine; Nle = norleucine; Hcy = homocysteine; Hhc = homohomocysteine; Pen = penicillamine; Aib = aminoisobutyric acid; Nal = 2-naphthylalanine; D-Nal = D-2-naphthylalanine; Ain = 2-aminoindan-2-carboxylic acid; Achxa = 4-amino-cyclohexylalanine; Amf = 4-aminomethyl-phenylalanine; Aec = S-(2-aminoethyl)cysteine; Apc = S-(3-aminopropyl)cysteine; Aes = O-(2-aminoethyl)serine; Aps = O-(3-aminopropyl)serine; Abu = 2-aminobutyric acid; Nva = norvaline; T(CH_2OH) = threoninol (on which the carboxylic acid moiety has been reduced to a primary alcohol); ϵ-K = a lysine residue in a peptide in which the peptide bond involves the ϵ-amino group on the lysine sidechain rather than the α-amino group; Pic = picolinoyl (pyridine-2-carbonyl); Pica = picolylamine (2-(aminomethyl)pyridine); BAT = N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; BAT acid (protected) = N^9-(*t*-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanoicacid; BAM = N^1,N^4-*bis*(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; BAM (protected) = N^1-(*t*-butoxycarbonyl)-N^1,N^4-*bis*(2-methyl-2-triphenylmethylthiopropyl)-1,4,10-triazadecane; [BAT-BM]=*N*-[2-(*N'*,*N'*-*bis*(2-maleimidoethyl)aminoethyl]-N^9-(*t*-butoxycarbonyl)-N^6,N^9-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; [BAT-BS] = *N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl]-N^6,N^9-*bis*(2-mercapto-2-methylpropyl)-6,9-diazanonanamide;

[BMME] = *bis*-maleimidomethylether; [BSME] = *bis*-succinimidomethylether; [DTPA] = diethylenetriaminepentaacetic acid.

EXAMPLE 3

Inhibition of Binding of [^{125}I-Tyr^{11}]somatostatin-14 to AR42J Rat Pancreatic Tumor Cell Membranes

The ability of various somatostatin analogues of the invention to bind to somatostatin receptors *in vitro* was demonstrated by assaying the ability of such analogues to inhibit binding of a radiolabeled somatostatin analogue to somatostatin receptor-containing cell membranes. The rat pancreatic tumor cell line AR42J which expresses the somatostatin receptor was cultured in Dulbecco's minimal essential media (DMEM) supplemented with 10% fetal bovine serum (FBS) and 8mM glutamine in a humdified 5% CO_2 atmosphere at 37°C in T-flasks. Harvested cells were homogenized in cold 50mM Tris-HCl buffer (pH 7.4) and the homogenate then centrifuged at 39,000*g* for 10min at 4°C. Pellets were washed once with buffer and then resuspended in an ice-cold solution of 10mM Tris-HCl (pH 7.4). Equal aliquots of this cell membrane preparation were incubated with [^{125}I-Tyr^{11}]somatostatin-14 (at a final concentration of 0.5nM and 750,000cpm/mL, at a specific activity of 2000Ci/mmol, Amersham, Arlington Heights, IL) and peptide or peptide-rhenium complex at a final concentration of from 10^{-11}M to 10^{-6}M in a solution of 50mM HEPES (pH 7.4) containing 1% bovine serum albumin (BSA), 5mM $MgCl_2$, Trasylol (200,000 International Units), bacitracin (0.02mg/mL) and phenylmethylsulfonylfluoride (0.02mg/mL) for 25min at 30°C. Using a filtration manifold, this mixture was filtered through a polyethyleneimine-washed GC/F filter (Whatman, Maidstone, England), and the residue remaining on the filter washed thrice with 5mL cold HEPES buffer. The filter and a sample of the filter washings were then counted in a gamma counter. To assess non-specific binding, the assay was performed in the presence of unlabeled somatostatin-14 at 200nM. Data analysis including Hill plots of the data provided inhibition constants (*see* Bylund & Yamamura, "Methods of receptor binding", *in* Methods in Neurotransmitter Receptor Analysis, Yamamura *et al.*, eds., Raven Press: New York, 1990).

These results are presented in the following Tables. The data show that the peptides of the instant invention have a high affinity of binding for somatostatin receptors.

TABLE II

[Re=O]-complexed Peptides	MH^+	K_i (nM)
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.K(ε-K)GC.amide)	1529	0.51
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.GGC.amide)	1330	0.59
CH_2CO.FFW$_D$KTFCC$_{Acm}$GC$_{Acm}$.amide	1480	0.67
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.(ε-K)GC.amide)	1401	0.92
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.CGC.amide)	1375	1.7
CH_2CO.FFW$_D$KTFC(ε-K)GC	1506	5.9

TABLE III

Peptide	K_i (nM)
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy	< 0.01
CH_2CO.FYW$_D$KTFC.amide	0.16
CH_2CO.FFW$_D$KTF.Hhc.amide	0.41
cyclo.CYW$_D$KVC	0.43
CH_2CO.FFW$_D$KTFC.amide	0.45
cyclo.(*N*-CH_3)F.YW$_D$KV.K.[BAT]	0.46
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.K(ϵ-K)GC.amide)	0.65
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.C$_{Acm}$GC$_{Acm}$.amide)	0.79
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.CGC.amide)	1.5
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.CGC)	1.8
CH_2CO.FFW$_D$KTFC.[BAM]	1.9
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.(ϵ-K)GC.amide)	2.0
cyclo.(*N*-CH_3)F.YW$_D$KV.Hcy(CH_2CO.GGC.amide)	2.4
cyclo.(*N*-CH_3)F.YW$_D$KV.E.[BAM]	2.6
CH_2CO.NFFW$_D$KTFTC	2.7
CH_2CO.FFW$_D$KTFC	4.0
CH_2CO.FFW$_D$KTFC(ϵ-K)GC.amide	5.2
CH_2CO.FFW$_D$KTFCC$_{Acm}$GC$_{Acm}$.amide	7.5
CH_2CO.FFW$_D$KTF.Hcy	9.8

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

What is claimed is:

1. A composition of matter comprising a somatostatin receptor-binding peptide having the formula:

only H in (10)

$$\begin{array}{l} R^1(CR^2)\text{-}[C(R^3R^4)]_m\text{-}CO\text{-}A^1A^2A^3B^1B^2B^3B^4C^1C^2C^3\text{-}NH\text{-}C(R^{11})\text{-}X^2 \\ \quad | \\ \quad Z \text{———} (CR^7R^8) \text{—————} (CR^5R^6)_p \end{array}$$

wherein R^1, R^2, R^5 and R^6 are independently H, lower alkyl or substituted alkyl, aryl or substituted aryl;

R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or wherein either R^3 or R^4 is X^1;

A^1 and C^3 are independently a bond or a D- or L-amino acid;

A^2, A^3 and C^1 are each independently a bond or a lipophilic D- or L-amino acid;

B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib;

C^2 is a bond or D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or substituted derivatives thereof;

X^1 is $N(R^{10})_2$, wherein each R^{10} is independently hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl or substituted with a hydrophilic moiety of less than about 1500 daltons;

X^2 is $-COOR^9$, $-CH_2OH$, CH_2COOR^9, or $-CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or substituted derivatives thereof, or substituted with a hydrophilic moiety of less than about 1500 daltons;

m is 0, 1, 2 or 3;

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

p is 0, 1 or 2;

R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 or R^8 are -COOH or $CO.N(R^{10})_2$ or $-COOR^{12}$, or R^7 and R^8 together comprise O;

R^{12} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl;

Z is S, O, NR^{13}, $NR^{13}NR^{13}$, $NR^{13}.CO.NR^{13}$, SO_2, $NR^{13}SO_2$ or S=O;

R^{13} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl;

wherein when Z is NR^{13}, R^7 and R^8 do not together comprise an oxygen atom;

or having the formula:

cyclo-A^4-$B^1B^2B^3B^4$-C^4-

wherein B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib;

C^4 is an L-amino acid having a sidechain comprising a mercapto group;

A^4 is a lipophilic D-amino acid or a lipophilic L-(α-*N*-alkyl) amino acid or L-proline or substituted derivatives thereof;

wherein the moiety is a cyclic peptide moiety having an amino terminus of A^4 and a carboxyl terminus of C^4 that are covalently linked.

2. The peptide of Claim 1 wherein X^1 is an amino acid or a peptide sequence comprising 10 or fewer amino acids, or a monosaccharide or oligosaccharide comprising 10 or fewer saccharide units, or a poly(*N*-carboxyalkyl)amine or a poly-oxy anion, and X^2 is a poly(*N*-carboxyalkyl)amine or with a polyoxy-anion, or an amino acid or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharaide or oligosaccharide comprising 10 or fewer saccharide units.

3. The somatostatin receptor-binding peptide of Claim 1 wherein B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

4. The composition of matter of Claim 1 further comprising a polyvalent linking

moiety that is covalently linked to a multiplicity of the somatostatin receptor-binding peptides to form a multimeric polyvalent somatostatin receptor binding agent, wherein the molecular weight of the multimeric polyvalent somatostatin receptor binding agent is less than about 20,000 daltons.

5. The reagent of Claim 4 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl)]-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylethyl)amine or a derivative thereof.

6. The composition of matter according to Claim 1 wherein the somatostatin receptor-binding peptide is chemically synthesized *in vitro*.

7. The composition of matter according to Claim 6 wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

8. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the somatostatin receptor binding peptide of Claim 1 to the animal.

9. The method of Claim 8 wherein the animal is a human.

10. A composition of matter comprising a reagent comprised of a somatostatin receptor-binding peptide having the formula:

$$\begin{array}{l} R^1(CR^2)\text{-}[C(R^3R^4)]_m\text{-CO-}A^1A^2A^3B^1B^2B^3B^4C^1C^2C^3\text{-NH-}CR^{11}\text{-}X^2 \\ \quad | \\ Z\text{---}(CR^7R^8)\text{------}(CR^5R^6)_p \end{array}$$

wherein R^1, R^2, R^5 and R^6 are independently H, lower alkyl or substituted alkyl, aryl or substituted aryl;

R^3 and R^4 are each independently H, lower alkyl or substituted alkyl, aryl or substituted aryl, or wherein either R^3 or R^4 is X^1;

A^1 and C^3 are independently a bond or a D- or L-amino acid;

A^2, A^3 and C^1 are each independently a bond or a lipophilic D- or L-amino acid;

B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib;

C^2 is a bond or D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or substituted derivatives thereof;

X^1 is $N(R^{10})_2$, wherein each R^{10} is independently hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl or substituted with a hydrophilic moiety of less than about 1500 daltons;*

X^2 is $-COOR^9$, $-CH_2OH$, CH_2COOR^9, or $-CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or substituted derivatives thereof, or substituted with a hydrophilic moiety of less than about 1500 daltons;

m is an integer that is 0, 1, 2 or 3;

p is an integer that is 0, 1 or 2;

R^7 and R^8 are independently H, lower alkyl or substituted lower alkyl, or either R^7 or R^8 are -COOH or $-CO.N(R^{10})_2$ or $-COOR^{12}$, or R^7 and R^8 together comprise O;

R^{12} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl;

Z is a bond, S, O, NR^{13}, $NR^{13}NR^{13}$, $NR^{13}.CO.NR^{13}$, SO_2, $NR^{13}SO_2$ or S=O;

R^{13} is hydrogen, lower alkyl or substituted lower alkyl, aryl or substituted aryl;

or having the formula:

$$cyclo\text{-}A^4\text{-}B^1B^2B^3B^4\text{-}C^4\text{-}$$

wherein B^1 is D- or L-Phe or D- or L-Tyr or D- or L-Nal or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 is Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva or Aib;

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

C^4 is an L-amino acid;

A^4 is a lipophilic D-amino acid or a lipophilic L-(α-*N*-alkyl) amino acid or L-cysteine or L-proline or substituted derivatives thereof;

wherein the moiety is a cyclic peptide moiety having an amino terminus of A^4 and a carboxyl terminus of C^4 that are covalently linked;

and wherein the somatostatin receptor-binding peptide is covalently linked to a radiolabel-binding moiety, wherein the radiolabel-binding moiety is not covalently linked to the moieties B^1, B^2, B^3, B^4 or A^4 of the peptide.

11. The reagent of Claim 10 wherein X^1 is an amino acid or a peptide sequence comprising 10 or fewer amino acids, or a monosaccharide or oligosaccharide comprising 10 or fewer saccharide units, or a poly(*N*-carboxyalkyl)amine or a poly-oxy anion and X^2 is a poly(*N*-carboxyalkyl)amine or a polyoxy-anion, or an amino acid or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide or oligosaccharide comprising 10 or fewer saccharide units.

12. The somatostatin receptor-binding peptide of Claim 10 wherein B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

13. The reagent of Claim 10 wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of the somatostatin receptor binding peptides and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent somatostatin receptor binding reagent, wherein the molecular weight of the multimeric polyvalent somatostatin receptor binding reagent is less than about 20,000 daltons.

14. The reagent of Claim 13 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl)]-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylethyl)amine or a derivative thereof.

15. A scintigraphic imaging agent comprising the reagent of Claim 10 radiolabeled with technetium-99m.

16. A scintigraphic imaging agent comprising the reagent of Claim 10 radiolabeled

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

with indium-111, gallium-67 or gallium-68.

17. A scintigraphic imaging agent comprising the somatostatin receptor binding peptide of Claim 1 radiolabeled with iodine-123 or iodine-125.

18. A radiotherapeutic agent comprising the reagent of Claim 10 radiolabeled with a cytotoxic radioisotope selected from the group consisting of scandium-47, copper-67, gallium-72, yttrium-90, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, and bismuth-212.

19. A radiotherapeutic agent comprising the somatostatin receptor binding peptide of Claim 1 radiolabeled with iodine-125, iodine-131 or astatine-131.

20. A complex formed by reacting the reagent of Claim 10 with technetium-99m in the presence of a reducing agent.

21. The complex of Claim 20, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

22. A complex formed by labeling the reagent of Claim 10 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

23. A composition of matter comprising the reagent of Claim 10 and a stannous ion.

24. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of Claim 10 and a sufficient amount of reducing agent to label the reagent with technetium-99m.

25. A method for labeling a reagent according to Claim 10 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

26. The method of Claim 25, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

27. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of the reagent of Claim 15 and detecting the technetium-99m localized at the site in the mammalian body.

28. The reagent according to Claim 10 wherein the somatostatin receptor-binding peptide is chemically synthesized *in vitro*.

29. The reagent according to Claim 28 wherein the somatostatin receptor-binding

peptide is synthesized by solid phase peptide synthesis.

30. The reagent according to Claim 28 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during *in vitro* chemical synthesis.

31. The reagent according to Claim 30 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.

32. The reagent of Claim 10 wherein the radiolabel-binding moiety having the formula:

$$C(pgp)^s\text{-}(aa)\text{-}C(pgp)^s$$

wherein $(pgp)^s$ is H or a thiol protecting group and (aa) is an amino acid;

$$A\text{-}CZ(B)\text{-}[C(R'R'')]_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC or R'''';

B is H, SH, -NHR''', -N(R''')-(peptide), or R'''';

X is H, SH, -NHR''', -N(R''')-(peptide) or R'''';

Z is H or R'''';

R', R'', R''' and R'''' are independently H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

and
where B is -NHR''' or -N(R''')-(peptide), X is SH, and n is 1 or 2;

where X is -NHR''' or -N(R''')-(peptide), B is SH, and n is 1 or 2;

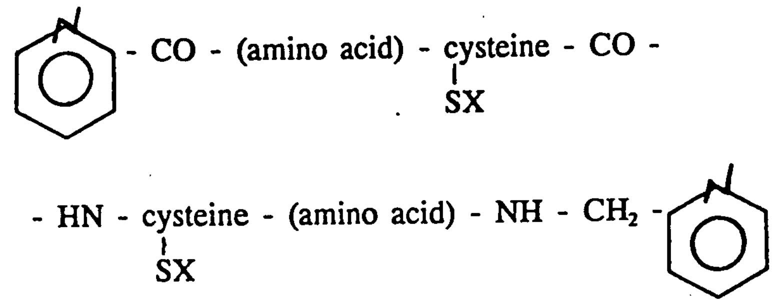
where B is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, X is SH, and n is 0 or 1;

where A is H or R'''', then where B is SH, X is -NHR''' or -N(R''')-(peptide) and where X is SH, B is -NHR''' or -N(R''')-(peptide);

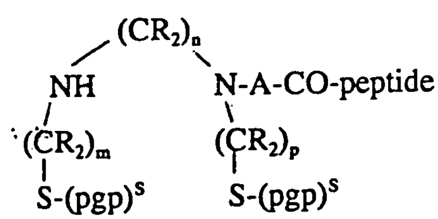
where X is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH; where Z is methyl, X is methyl, A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, B is SH and n is 0;

where B is SH and X is SH, n is not 0;

and wherein the thiol moiety is in the reduced form;

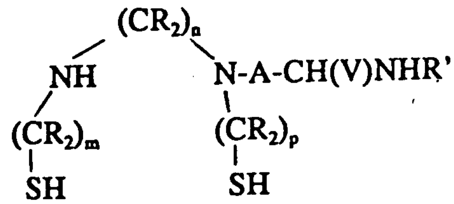


wherein X = H or a protecting group;
(amino acid) = any amino acid;



wherein each R is independently H, CH_3 or C_2H_5;
each $(pgp)^s$ is independently a thiol protecting group or H;
m, n and p are independently 2 or 3;
A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;

or



wherein each R is independently H, CH_3 or C_2H_5;

m, n and p are independently 2 or 3;

A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;

V = H or -CO-peptide;

R' = H or peptide;

and wherein when V = H, R' = peptide and when R' = H, V = -CO-peptide; wherein each R is independently H, lower alkyl having 1 to 6 carbon atoms, phenyl, or phenyl substituted with lower alkyl or lower alkoxy, and wherein each n is independently 1 or 2

33. The reagent of Claim 32 wherein the cysteine of the radiolabel-binding moiety having formula

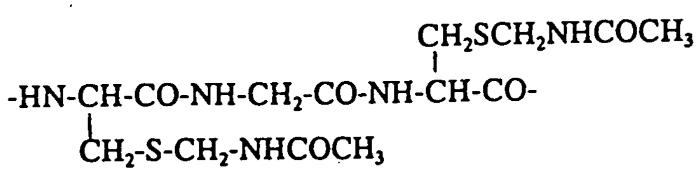
$$C(pgp)^{s}\text{-}(aa)\text{-}C(pgp)^{s}$$

has a protecting group of the formula

$$\text{-}CH_2\text{-}NH\text{-}CO\text{-}R$$

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-,3-,4-pyridyl, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl.

34. The reagent of Claim 32 wherein the radiolabel-binding moiety $C(pgp)^{s}$-(aa)-$C(pgp)^{s}$ has the formula:



35. A scintigraphic imaging agent that is the reagent of Claim 32 radiolabeled with technetium-99m.

36. A complex formed by reacting the reagent of Claim 32 with technetium-99m in the presence of a reducing agent.

37. The complex of Claim 36, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

38. A complex formed by labeling the reagent of Claim 32 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

39. A composition of matter comprising the reagent of Claim 32 and a stannous ion.

40. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of Claim 32 and a sufficient amount of reducing agent to label the reagent with technetium-99m.

41. A method for labeling a reagent according to Claim 32 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

42. The method of Claim 41, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

43. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of the reagent of Claim 35 and detecting the technetium-99m localized at the site in the mammalian body.

44. The reagent according to Claim 32 wherein the somatostatin receptor-binding peptide is chemically synthesized *in vitro*.

45. The reagent according to Claim 44 wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

46. The reagent according to Claim 44 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during *in vitro* chemical synthesis.

47. The reagent according to Claim 46 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.

48. The reagent of Claim 32 wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of the somatostatin receptor binding peptides and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent somatostatin receptor binding reagent, wherein

the molecular weight of the multimeric polyvalent somatostatin receptor binding reagent is less than about 20,000 daltons.

49. The reagent of Claim 48 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-[2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl)]-N^6,N^9-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylethyl)amine or a derivative thereof.

50. A composition of matter comprising the peptide of Claim 1 selected from the group consisting of somatostatin receptor binding peptides having the formula:

cyclo-(*N*-CH_3)FYW_DKV.Hcy

CH_2CO.FYW_DKTFC.amide

CH_2CO.FYW_DKTF.Hhc.amide

cyclo-CYW_DKVC

CH_2CO.FFW_DKTFC.amide

CH_2CO.FFW_DKTFC

cyclo-(*N*-CH_3)FYW_DKV.K

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.GGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.K.(ε-K).GC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.(ε-K).GC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.C_{Acm}GC_{Acm}.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.CGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.GGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.CGC)

cyclo-(*N*-CH_3)FYW_DKV.E

CH_2CO.NFFW_DKTFTC

CH$_2$CO.FFW$_D$KTFC

CH$_2$CO.FFW$_D$KTFC.(ϵ-K).GC.amide

CH$_2$CO.FFW$_D$KTFC.C$_{Acm}$GC$_{Acm}$.amide

CH$_2$CO.FFW$_D$KTF.Hcy

CH$_2$CO.YW$_D$KTC

CH$_2$CO.YW$_D$KT.Hcy.amide

CH$_2$CO.YW$_D$KT.Hhc.T(CH$_2$OH)

CH$_2$CO.YW$_D$KTCTGGC$_{Mob}$.amide

CH$_2$CO.YW$_D$KTF.Hhc

D-*phenyl*-CH$_2$CHCO.YW$_D$KTC

CH$_2$CO.FW$_D$KT.Pen

CH$_2$CO.FW$_D$KT.Hcy

CH$_2$CO.FW$_D$KT.Hcy.amide

CH$_2$CO.YW$_D$KTC.amide

CH$_2$CO.YW$_D$KTCT

CH$_2$CO.YW$_D$KTCT(CH$_2$OH)

CH$_2$CO.YW$_D$KTCT.C$_{Acm}$GC$_{Acm}$.amide

CH$_2$CO.FW$_D$KTC$_D$

CH$_2$CO.FW$_D$KTC

51. A composition of matter comprising the reagent of Claim 10 selected from the group consisting of reagents having the formula:

cyclo-CYW$_D$KVC

CH$_2$CO.FFW$_D$KTFC.[BAM]

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

cyclo-(*N*-CH_3)FYW_DKV.K.[BAT]

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.GGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.K.(ϵ-K).GC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.(ϵ-K).GC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.C_{Acm}GC_{Acm}.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.CGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.GGC.amide)

cyclo-(*N*-CH_3)FYW_DKV.Hcy(CH_2CO.CGC)

cyclo-(*N*-CH_3)FYW_DKV.E.[BAM]

CH_2CO.FFW_DKTFC.(ϵ-K).GC.amide

CH_2CO.FFW_DKTFC.C_{Acm}GC_{Acm}.amide

CH_2CO.YW_DKTCTGGC_{Mob}.amide

CH_2CO.YW_DKTCT.C_{Acm}GC_{Acm}.amide

52. A radiotherapeutic agent that is the reagent of Claim 32 radiolabeled with rhenium-186 or rhenium-188 in the presence of a reducing agent.

53. The radiolabeled radiotherapeutic agent of Claim 52 wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and an ferrous ion.

54. A kit for preparing a radiopharmaceutical preparation of the radiotherapeutic agent comprising a sealed vial containing an amount of the reagent of Claim 32 and a sufficient amount of a reducing agent to radiolabel the reagent with rhenium-186 or rhenium-188.

55. The composition of matter of Claim 51 radiolabeled with a radioisotope selected from the group consisting of gallium-68, technetium-99m, indium-111, and iodine-123.

56. The composition of matter of Claim 51 radiolabeled with a radioisotope selected from the group consisting of scandium-47, copper-67, gallium-72, yttrium-90, iodine-125,

iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, astatine-211, bismuth-212 and astatine-131.

57. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the composition of matter of Claim 50 to the animal.

58. The method of Claim 57 wherein the animal is a human.

59. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the composition of matter of Claim 56 to the animal.

60. The method of Claim 59 wherein the animal is a human.

61. The method of Claim 59 wherein the therapeutically effective amount of the composition administered to the animal is an amount from about 10 to about 200 milliCuries of the radiolabeled composition.

62. A pharmaceutical composition comprising the radiolabeled radiotherapeutic of Claim 56 in a pharmaceutically acceptable carrier.

63. A composition of matter comprising a complex formed by reacting the reagent of Claim 10 with a non-radioactive metal.

64. The complex of Claim 63 wherein the non-radioactive metal is rhenium.

65. A composition of matter comprising a complex formed by reacting the reagent of Claim 13 with a non-radioactive metal.

66. A composition of matter comprising a complex formed by reacting the scintigraphic imaging agent of Claim 15 with a non-radioactive metal.

67. A composition of matter comprising a complex formed by reacting the scintigraphic imaging agent of Claim 16 with a non-radioactive metal.

68. A composition of matter comprising a complex formed by reacting the scintigraphic imaging agent of Claim 17 with a non-radioactive metal.

69. A composition of matter comprising a complex formed by reacting the radiotherapeutic agent of Claim 18 with a non-radioactive metal.

70. A composition of matter comprising a complex formed by reacting the radiotherapeutic agent of Claim 19 with a non-radioactive metal.

71. The reagent of Claim 10 radiolabeled with technetium-99m, indium-111, gallium-67 or gallium-68.

72. The peptide of Claim 1 radiolabeled with iodine-123, iodine-125, iodine-131 or astatine-211.

73. The reagent of Claim 10 radiolabeled with a radioisotope selected from the group consisting of scandium-47, copper-67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, and bismuth-212.

74. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of the reagent of Claim 10 radiolabeled with a detectable radioisotope and detecting the radioisotope localized at the site in the mammalian body.

75. The peptide of Claim 51 radiolabeled with iodine-123, iodine-125, iodine-131 or astatine-211.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

ABSTRACT OF THE DISCLOSURE

This invention relates to therapeutic reagents and peptides, including radiotherapeutic reagents and peptides, radiodiagnostic reagents and peptides, and methods for producing labeled radiodiagnostic agents. Specifically, the invention relates to cyclic peptide derivatives and analogs of somatostatin, and embodiments of such peptides radiolabeled with a radioisotope, as well as methods and kits for making, radiolabeling and using such peptides for radiodiagnostic and radiotherapeutic purposes. The invention specifically relates to cyclic peptide derivatives and analogues of somatostatin radiolabeled with technetium-99m and uses thereof as scintigraphic imaging agents. The invention also specifically relates to cyclic peptide derivatives and analogues of somatostatin radiolabeled with cytotoxic radioisotopes such as rhenium-186 (^{186}Re) and rhenium-188 (^{188}Re) for use as radiotherapeutic agents. Methods and kits for making, radiolabeling and using such peptides diagnostically and therapeutically in a mammalian body are also provided.

ALLEGRETTI & WITCOFF, LTD
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000

D

US005620675A

United States Patent [19]

McBride et al.

[11] Patent Number: 5,620,675

[45] Date of Patent: *Apr. 15, 1997

[54] RADIOACTIVE PEPTIDES

[75] Inventors: William McBride, Manchester; Richard T. Dean, Bedford, both of N.H.

[73] Assignee: Diatech, Inc., Londonderry, Ill.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,552,525.

[21] Appl. No.: 95,760

[22] Filed: Jul. 21, 1993

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 902,935, Jun. 23, 1992.

[51] Int. Cl.[6] A61K 38/31; A61K 38/00; A61K 51/00

[52] U.S. Cl. 424/1.69; 530/500; 530/322; 530/311; 424/1.73; 424/9.3; 424/9.35; 424/9.4; 424/9.43; 424/9.6

[58] Field of Search 530/311, 313, 530/303, 333, 330, 350, 326, 402, 317, 300, 322; 424/1.11, 1.1, 1.69, 1.73, 9.3, 9.35, 9.4, 9.43, 9.6

[56] References Cited

U.S. PATENT DOCUMENTS

4,235,886	11/1980	Freidinger	530/326
4,444,690	4/1984	Fritzberg	530/26
4,472,509	9/1984	Gansow et al.	530/326
4,485,101	11/1984	Coy et al.	530/326
4,608,251	8/1986	Mia	530/313
4,611,054	9/1986	Freidinger	530/326
4,612,366	9/1986	Nutt	530/326
4,853,371	8/1989	Coy et al.	530/326
4,871,717	10/1989	Coy et al.	530/326
4,904,642	2/1990	Coy et al.	530/326
5,073,541	12/1991	Taylor et al.	530/326
5,084,442	1/1992	Felix et al.	530/317
5,196,510	3/1993	Rodwell et al.	530/326
5,212,072	5/1993	Blalock et al.	530/333
5,225,180	7/1993	Dean et al.	530/350

FOREIGN PATENT DOCUMENTS

83111747	7/1984	European Pat. Off. .
85810617	7/1986	European Pat. Off. .
87300426	9/1987	European Pat. Off. .
90302760	3/1990	European Pat. Off. .
90304551	10/1990	European Pat. Off. .
911048445.2	10/1991	European Pat. Off. .
92810381.1	11/1992	European Pat. Off. .
8927255	6/1990	United Kingdom .
2225579	6/1990	United Kingdom .

OTHER PUBLICATIONS

Reubi et al, Chem Abstracts, 103: 135620 (1985).

Bomanji et al., 1992, "A scintigraphic comparison of iodine–123–metaiodobenzylguanidine and an iodine–labeled somatostatin analog (Tyr–3–octreotide) in metastatic carcinoid tumors", J. Nucl. Med. 33: 1121–1124.

Krenning et al., 1992, "Somatostatin receptor scintigraphy with indium–111–DTPA–D–Phe–1–octreotide in man: Metabolism, dosimetry and comparison with iodine–123–Tyr–3–octreotide", J. Nucl. Med. 33: 652–658.

Cox et al., 1991, "Technetium Labeled Somatostatin: A Potential Agent for In Vivo Tumour Localization", Abstract, 7th International Symposium on Radiopharmacology, p. 16.

Albert et al., 1991, "A Somatostatin Analogue to Image SS–Receptor–Positive Tumours: [^{111}In–DTPA–DPhe] 1^{1}–Octreotide (SDZ 215–811)", Abstract LM10, 12th American Peptide Symposium: 1991.

Faglia et al., 1991, "In vivo detection of somatostatin receptors in patients with functionless pituitary adenomas by means of a radioiodinated analog of somatostatin ([^{123}I])SDZ 204–090)", J. Clin. Endocrinol. Metab. 73: 850–856.

Larson, 1991, "Receptors on Tumors Studied with Radionuclide Scintigraphy", J. Nucl. Med. 32: 1189–1191.

Primary Examiner—John Kight
Assistant Examiner—Louise N. Leary
Attorney, Agent, or Firm—Banner & Witcoff, Ltd.

[57] ABSTRACT

This invention relates to therapeutic reagents and peptides, radiodiagnostic reagents and peptides, and methods for producing label radiodiagnostic agents. Specifically, the invention relates to linear peptide derivatives and analogs of somatostatin, and embodiments of such peptides radiolabeled with a radioisotope, as well as methods and kits for making, radiolabeling and using such peptides for radiodiagnostic and radiotherapeutic purposes. The invention specifically relates to linear peptide derivatives and analogues of somatostatin radiolabeled with technetium-99m and uses thereof as scintigraphic imaging agents. The invention so specifically relates to liner peptide derivatives and analogues of somatostatin radiolabeled with cytotoxic radioisotopes such as rhenium-186 (^{186}Re) and rhenium-188 (^{188}Re) for use as radiotherapeutic agents. Methods and kits for making, radiolabeling and using such peptides diagnostically and therapeutically in a mammalian body are also provide.

68 Claims, No Drawings

RADIOACTIVE PEPTIDES

This application is a continuation-in-part of copending U.S. patent application Ser. No. 07/902,935, filed Jun. 23, 1992.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to therapeutic agents and peptides, radiotherapeutic agents and peptides, radiodiagnostic agents and peptides, and methods for producing such labeled radiodiagnostic and radiotherapeutic agents. Specifically, the invention relates to linear peptide derivatives and analogues of somatostatin, and embodiments of such peptides labeled with gamma radiation-emitting radioisotopes such as technetium-99m (Tc-99m), as well as methods and kits for making, radiolabeling and using such peptides to image sites in a mammalian body. The invention also relates to linear peptide derivatives and analogues of somatostatin labeled with cytotoxic radioisotopes such as rhenium-186 (^{186}Re) and rhenium-188 (^{188}Re), and methods and kits for making, radiolabeling and using such peptides therapeutically in a mammalian body.

2. Decription of the Prior Art

Somatostatin is a tetradecapeptide that is endogenously produced by the hypothalamus and pancreas in humans and other mammals. The peptide has the formula:

Brady, European Patent Application No. 83111747.8 discloses dicyclic hexapeptide somatostatin analogues useful in the treatment of a number of human diseases.

Bauer et al., European Patent Application No. 85810617.2 disclose somatostatin derivatives useful in the treatment of a number of human diseases.

Eck and Moreau, European Patent Application No. 90302760.5 disclose therapeutic octapeptide somatostatin analogucs.

Coy and Murphy, European Patent Application Ser. No. 90304551.6 disclose linear somatostatin analogues.

Coy and Murphy, International Patent Application Ser. No. PCT/US90/07074 disclose somatostatin analogues for therapeutic uses.

Schally et al., European Patent Application Ser. No. EPA 911048445.2 disclose cyclic peptides for therapeutic use.

Bodgen and Moreau, International Patent Application Ser. No. PCT/US92/01027 disclose compositions and methods for treating proliferative skin disease.

Somatostatin exerts it effects by binding to specific receptors expressed at the cell surface of cells comprising the central nervous system, the hypothalamus, the pancreas, and the gastrointestinal tract. These high-affinity somatostatin binding sites have been found to be abundantly expressed at the cell surface of most endocrine-active tumors arising from these tissues. Expression of high-affinity binding sites for somatostatin is a marker for these tumor cells, and specific binding with somatostatin can be exploited to locate and identify tumor cells in vivo.

Ala—Gly—Cys—Lys—Asn—Phe—Phe—Trp—Lys—Thr—Phe—Thr—Ser—Cys   Formula I

[Single letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d ed.), 1988, (MacMillan Publishing: New York), p.33]. This peptide exerts a wide variety of biological effects in vivo. It is known to act physiologically on the central nervous system, the hypothalamus, the pancreas, and the gastrointestinal tract.

Somatostatin inhibits the release of insulin and glucagon from the pancreas, inhibits growth hormone release from the hypothalamus, and reduces gastric secretions. Thus, somatostatin has clinical and therapeutic applications for the alleviation of a number of ailments and diseases, both in humans and other animals. Native somatostatin is of limited utility, however, due to its short half-life in vivo, where it is rapidly degraded by peptidases. For this reason, somatostatin analogues having improved in vivo stability have been developed in the prior art.

Freidinger, U.S. Pat. No. 4,235,886 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Coy and Murphy, U.S. Pat. No. 4,485,101 disclose synthetic dodecapeptide somatostatin analogues.

Freidinger, U.S. Pat. No. 4,611,054 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Nutt, U.S. Pat. No. 4,612,366 disclose cyclic hexapeptide somatostatin analogues useful in the treatment of a number of diseases in humans.

Coy et al., U.S. Pat. No. 4,853,371 disclose synthetic octapeptide somatostatin analogues.

Coy and Murphy, U.S. Pat. No. 4,871,717 disclose synthetic heptapeptide somatostatin analogues.

Coy et al., U.S. Pat. No. 4,904,642 disclose synthetic octapeptide somatostatin analogues.

Taylor et al., U.S. Pat. No. 5,073,541 disclose a method of treating small cell lung cancer.

Methods for radiolabeling somatostatin analogues that have been modified so as to contain a tyrosine amino acid (Tyr or Y) are known in the prior art.

Albert et al., UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{123}I.

Bakker et al., 1990, J. Nucl. Med. 31: 1501–1509 describe radioactive iodination of a somatostatin analog and its usefulness in detecting tumors in vivo.

Bakker et al., 1991, J. Nucl. Med. 32: 1184–1189 teach the usefulness of radiolabeled somatostatin for radioimaging in vivo.

Bomanji et al., 1992, J. Nucl. Med. 33: 1121–1124 describe the use of iodinated (Tyr-3) octreotide for imaging metastatic carcinoid tumors.

Alternatively, methods for radiolabeling somatostatin by covalently modifying the peptide to contain a radionuclide-chelating group have been disclosed in the prior art.

Albert et al., UK Patent Application 8927255.3 disclose radioimaging using somatostatin derivatives such as octreotide labeled with ^{111}In via a chelating group bound to the amino-terminus.

Albert et al., European Patent Application No. WO 91/01144 disclose radioimaging using radiolabeled peptides related to growth factors, hormones, interferons and cytokines and comprised of a specific recognition peptide covalently linked to a radionuclide chelating group.

Albert et al., European Patent Application No. 92810381.1 disclose somatostatin peptides having amino-terminally linked chelators.

Faglia et al., 1991, J. Clin. Endocrinol. Metab. 73: 850–856 describe the detection of somatostatin receptors in patients.

Kwekkeboom et al., 1991, J. Nucl. Med. 32: 981 Abstract #305 relates to radiolabeling somatostatin analogues with ^{111}In.

Albert et al., 1991, Abstract LM10, 12th American Peptide Symposium: 1991 describe uses for ^{111}In-labeled diethylene-triaminopentaacetic acid-derivatized somatostatin analogues.

Krenning et al., 1992, J. Nucl. Med. 33: 652–658 describe clinical scintigraphy using [^{111}In][DTPA]octreotide.

These methods can be readily adapted to enable detection of tumor cells in vivo by radioimaging, based on the expression of high affinity binding sites for somatostatin on tumor cells. Radionuclides which emit gamma radiation can be readily detected by scintigraphy after injection into a human or an animal. A variety of radionuclides are known to be useful for radioimaging, including ^{67}Ga, ^{68}Ga, ^{99m}Tc (Tc-99m), ^{111}In, ^{123}I or ^{125}I. The sensitivity of imaging methods using radioactively-labeled peptides is much higher than other techniques known in the art, since the specific binding of the radioactive peptide concentrates the radioactive signal over the cells of interest, for example, tumor cells. This is particularly important for endocrine-active gastrointestinal tumors, which are usually small, slow-growing and difficult to detect by conventional methods. Labeling with technetium-99m (Tc-99m) is advantageous because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. Tc-99m has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator. Other radionuclides have effective half-lives which are much longer (for example, ^{111}In, which has a half-life of 60–70 h) or are toxic (for example, ^{125}I). Although Tc-99m is an ideal radiolabeling reagent, it has not been widely used in the art prior to the present invention [see, for example, Lamberts, J. Nucl. Med. 32: 1189–1191 (1991)].

Somatostatin and radiolabeled somatostatin analogues can also be used therapeutically. For these applications, cytotoxic radioisotopes are advantageous, such as scandium-47, copper67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, astatine-211, and bismuth-212. The rhenium isotopes ^{186}Re and ^{188}Re are particularly advantageous.

The use of chelating agents for radiolabeling proteins are known in the prior art, and methods for labeling peptides Tc-99m are disclosed in U.S. patent application Ser. Nos. 07/653,012 filed Feb. 8, 1991, now abandoned; 07/757,470 filed Sep. 10, 1991, now U.S. Pat. No. 5,225,180; 07/807,062 filed Nov. 27, 1991, now U.S. Pat. No. 5,443,815; 07/851,074 filed Mar. 13, 1992, now abandoned; 07/886,752 filed May 21, 1992, now abandoned; 07/893,981 filed Jun. 5, 1992, now U.S. Pat. No. 5,508,020; 07/955,466 filed Oct. 2, 1992, now abandoned; 07/977,628 filed Nov. 17, 1992, now U.S. Pat. No. 5,405,597; 08/044,825 filed Apr. 8, 1993, now abandoned; and co-pending application Ser. Nos. 07/871,282 filed Apr. 30, 1992; 08/019,864 filed Feb. 19, 1993; and Ser. No. 08/073,577 filed Jun. 7, 1993 and PCT International Applications PCT/US92/00757, PCT/US92/10716, PCT/US93/02320, PCT/US93/03687, PCT/US93/04794, and PCT/US93/06029, which are hereby incorporated by reference.

Fritzberg, U.S. Pat. No. 4,444,690 describes a series of technetium-chelating agents based on 2,3-bis(mercaptoacetamido) propanoate.

Gansow et al., U.S. Pat. No. 4,472,509 teach methods of manufacturing and purifying Tc-99m chelate-conjugated monoclonal antibodies.

Reno and Bottino, European Patent Application 87300426.1 disclose radiolabeling antibodies with Tc-99m.

Pak et al., European Patent Application No. WO 88/07382 disclose a method for labeling antibodies with Tc-99m.

Cox, International Patent Application No. PCT/US92/04559 discloses radiolabeled somatostatin derivatives containing two cysteine residues.

Rhodes, 1974, Sem. Nucl. Med. 4: 281–293 teach the labeling of human serum albumin with technetium-99m.

Khaw et al., 1982, J. Nucl. Med. 23: 1011–1019 disclose methods for labeling biologically active macromolecules with Tc-99m.

Byrne and Tolman, supra, disclose a bifunctional thiolactone chelating agent for coupling Tc-99m to biological molecules.

Cox et al., 1991, Abstract, 7th International Symposium on Radiopharmacology, p. 16, disclose the use of, Tc-99m-, ^{131}I- and ^{111}In-labeled somatostatin analogues in radiolocalization of endocrine tumors in vivo by scintigraphy.

Methods for directly labeling somatostatin, derivatives of somatostatin, analogues of somatostatin or peptides that bind to the somatostatin receptor and contain at least 2 cysteine residues that form a disulfide or wherein the disulfide is reduced to the sulfhydryl form, are disclosed in co-pending U.S. patent application Ser. No. 07/807,062, filed Nov. 27, 1991, now U.S. Pat. No. 5,443,815 which is hereby incorporated by reference.

There remains a need for synthetic (to make routine manufacture practicable and to ease regulatory acceptance) somatostatin analogues having increased in vivo stability, to be used therapeutically, as scintigraphic agents when radiolabeled with Tc-99m or other detectable radioisotopes for use in imaging tumors in vivo, and as radiotherapeutic agents when radiolabeled with a cytotoxic radioisotope such as rhenium-188. Small synthetic somatostatin analogues are provided by this invention that specifically fulfill this need.

SUMMARY OF THE INVENTION

The present invention provides somatostatin analogues that are linear peptides for therapeutic applications, including radiotherapeutic applications, and diagnostic applications, including radiodiagnostic applications, in particular scintigraphic imaging applications. Distinct from native somatostatin and somatostatin analogues known in the prior art, the linear peptides of the invention are not constrained within a cyclic structure. The invention also provides linear peptide reagents comprised of the linear peptide somatostatin analogues of the invention, wherein such peptides are covalently linked to a radiolabel-binding moiety. The invention provides such linear peptides, linear peptide reagents and radiolabeled linear peptide reagents that are scintigraphic imaging agents, radiodiagnostic agents and radiotherapeutic agents. Scintigraphic imaging agents of the invention comprise linear peptide reagents radiolabeled with a radioisotope, preferably technetium-99m. Radiotherapeutic agents of the invention comprise linear peptide reagents radiolabeled with a cytotoxic radioisotope, preferably rhenium-186 or rhenium-188. Methods for making and using such linear peptides, linear peptide reagents and radiolabeled embodiments thereof are also provided.

The present invention also provides scintigraphic imaging agents comprised of a linear peptide that is a somatostatin analogue and that is labeled with iodine-123, iodine-125 or iodine-131. Similarly, the invention provides alternative embodiments of the linear somatostatin peptide analogues radiolabeled with iodine-125, iodine-131 or astatine-211 for use as therapeutic agents.

The somatostatin analogues provided by the invention are somatostatin-receptor binding peptides having the following formula:

$$X^1\text{-}A^1A^2\text{-}B^1B^2B^3B^4\text{-}C^1C^2\text{-}X^2 \quad \text{(Formula II)}$$

wherein X^1 is a hydrophilic moiety which is not greater than 1500 Daltons in formula weight; A^1, A^2 and C^1 are each independently a lipophilic D- or L-amino acid, S-alkylated cysteine, penicillamine (Pen), homocysteine (Hcy) or homohomocysteine (Hhc; 3-mercaptopropyl) glycine; B^1 is D- or L-Phe, or D- or L-Tyr, or D- or L-2-naphthylalanine (Nal), or 2-amino-indane-2-carboxylic acid (Ain) or substituted derivatives thereof; B^2 is D- or L-Trp or substituted derivatives thereof; B^3 is D- or L-Lys, or homolysine (Hly), 4-aminocyclohexylalanine (Achxa), 4-aminomethyl-phenylalanine (Amf), S-(2-aminoethyl)cysteine (Aec), S-(3-aminopropyl)cysteine (Apc), O-(2-aminoethyl) serine (Aes), O-(3-aminopropyl)serine (Aps) or substituted derivatives thereof; B^4 is Thr, Ser, Val, Phe, Leu, Ile, 2-amino-isobutyric acid (Aib), 2-aminobutyric acid (Abu), norvaline (Nva), or norleucine (Nle); C^2 is D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or substituted derivatives thereof; X^2 is a hydrophilic moiety which is not more than 1500 Daltons in formula weight. In a preferred embodiment, X^1 is a hydrophilic moiety that comprises an amino acid, or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide, or an oligosaccharide comprising 10 or fewer saccharide units, or a poly(N-carboxyalkyl)amine, or a polyoxyanion. In another preferred embodiment, X^2 is a hydrophilic moiety that comprises a poly(N-carboxyalkyl)amine or polyoxyanion, or an amino acid, or a peptide having an amino acid sequence of no more than 10 residues (including peptides wherein the carboxyl group of the carboxyl-terminal amino acid is reduced to an alcohol), or a monosaccharide or an oligosaccharide comprising 10 or fewer saccharide units. In another preferred embodiment, B^1 is phenylalanine or tyrosine, B^2 is tryptophan, most preferably D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

The invention also provides linear peptide reagents comprising a linear peptide of Formula II covalently linked to a radiolabel-binding moiety, wherein X^1 is H, lower alkyl or substituted alkyl, aryl or substituted aryl, alkanoyl or substituted alkanoyl, aroyl or substituted aroyl, or a hydrophilic moiety which is not greater than 1500 Daltons in formula weight; A^1, A^2 and C^1 are each independently a lipophilic D- or L-amino acid, S-alkylated cysteine, penicillamine (Pen), homocysteine (Hcy) or homohomocysteine (Hhc; 3-mercaptopropyl) glycine; B^1 is D- or L-Phe, or D- or L-Tyr, or D- or L-2-naphthylalanine (Nal), or 2-amino-indane-2-carboxylic acid (Ain) or substituted derivatives thereof; B^2 is D-or L-Trp or substituted derivatives thereof; B3 is D- or L-Lys, or homolysine (Hly), 4-aminocyclohexylalanine (Achxa), 4-aminomethyl-phenylalanine (Amf), S-(2-aminoethyl)cysteine (Aec), S-(3-aminopropyl)cysteine (Apc), O-(2-aminoethyl) serine (Aes), O-(3-aminopropyl)serine (Aps) or substituted derivatives thereof; B^4 is Thr, Set, Val, Phe, Leu, Ile, 2-amino-isobutyric acid (Aib), 2-aminobutyric acid (Abu), norvaline (Nva), or norleucine (Nle); C^2 is D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nat or Aib or substituted derivatives thereof; X^2 is —$COOR^9$, —CH_2OH, CH_2COOR^9, or —$CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or substituted derivatives thereof, or substituted with a hydrophilic moiety which is not more than 1500 Daltons in formula weight. In a preferred embodiment, when Xis a hydrophilic moiety that moiety comprises an amino acid, or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide, or an oligosaccharide comprising 10 or fewer saccharide units, or a poly(N-carboxyalkyl)amine, or a polyoxyanion. In another preferred embodiment, when X^2 is a hydrophilic moiety that moiety comprises a poly(N-carboxyalkyl)amine or polyoxyanion, or an amino acid, or a peptide having an amino acid sequence of no more than 10 residues (including peptides wherein the carboxyl group of the carboxyl-terminal amino acid is reduced to an alcohol), or a monosaccharide or an oligosaccharide comprising 10 or fewer saccharide units. In another preferred embodiment, Bis phenylalanine or tyrosine, B^2 is tryptophan, most preferably D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

The present invention provides peptides that are linear somatostatin peptide analogues as described herein having increased in vivo stability compared with native somatostatin, and that are therapeutically useful in the alleviation of diseases or other ailments in humans or other animals.

The invention also provides scintigraphic imaging agents comprising the linear peptide reagents of the invention wherein the radiolabel-binding moiety is stably complexed with a radioisotope. In one such embodiment is provided a scintigraphic imaging agent wherein the linear somatostatin peptide analogue reagents of the invention are radiolabeled with technetium-99m. In other embodiments of the scintigraphic imaging agents of the invention the radioisotope is indium-111 or gallium-68. In still other embodiments, the scintigraphic imaging agents of the invention are linear peptides that are radiolabeled with iodine-123 or iodine-125.

The invention also provides radiotherapeutic agents that are the linear peptide reagents of the invention radiolabeled with a cytotoxic radioisotope that is selected from the group consisting of scandium-47, copper-67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium-165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188,astatine-211 and bismuth-212. In preferred embodiments, the radioisotope is rhenium-186 or rhenium-188. In additional preferred embodiments, the cyclic peptides of the invention are radiolabled with iodine-125, iodine-131 or astatine-211.

In another embodiment, the invention provides therapeutic agents comprising the linear spmatostain analogue peptide reagents of the invention complexed with a non-radioactive metal such as rhenium. Combination embodiments, wherein such a complex is also radiolabeled, either directly or via a radiolabel-binding moiety, are also provided by the invention and are within its scope.

The invention also provides pharmaceutical compositions comprising the somatostatin receptor-binding peptides of the invention in a pharmaceutically acceptable carrier.

The invention also provides a method for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the somatostatin analogues of the invention to the animal. In preferred embodiments, the amount of the somatostatin analogue administered is from about 0.1 to about 50 mg/kg body weight/day.

It is an advantage of the somatostatin analogues provided by this invention that the peptides retain high affinity for somatostatin receptors even though they are linear peptides. As the preferred embodiments lack intramolecular disulfide bonding, the advantageous feature of the linear somatostatin peptide analogues of this invention is that their stability is not dependent on the formation or persistence of intramolecular disulfide bonds. This feature is in turn advantageous

because the high affinity of the peptides of this invention for somatostatin receptors is thus not a function of the integrity of labile intramolecular crosslinks such as disulfide bonds. Additionally, the peptide reagents of the invention retain their high affinity for somatostatin receptors after being subjected to radiolabeling via covalently-linked radiolabeled binding moieties. In contrast, for example, Tc-99m conjugation to a Tc-99m binding moiety covalently linked to native somatostatin, or to a somatostatin analogue having a disulfide bond, can result in reduction of the disulfide accompanied by a loss of biological activity. Such loss of biological activity can also occur in vivo using native somatostatin, or to any somatostatin analogue having a disulfide bond. The present invention is not subject to similar losses in biological activity in vivo because the somatostatin analogues of the invention are active as linear peptides.

A first aspect of the reagents provided by the invention for preparing radiolabeled agents of the invention are reagents, each comprised of a peptide that is a somatostatin analogue that is covalently linked to a radiolabel-binding moiety having formula:

$$C(pgp)^s\text{-}(aa)\text{-}C(pgp)^s$$

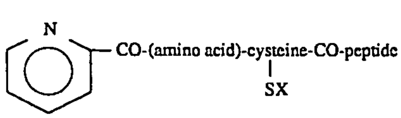
wherein $(pgp)^s$ is H or a thiol protecting group and (aa) is an amino acid. In a preferred embodiment, the amino acid is glycine. In another preferred embodiment, the agent is a scintigraphic imaging agent. In yet another preferred embodiment, the agent is a radiotherapeutic agent.

In a second embodiment, the invention provides peptide reagents capable of being radiolabeled for use as scintigraphic imaging agents or radiotherapeutic agents, each comprising a somatostatin analogue that is covalently linked to a radiolabel-binding moiety of formula:

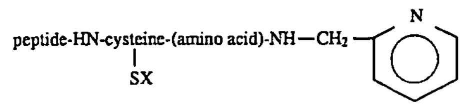
$$A\text{-}CZ(B)\text{-}[C(R'R'')]_n\text{-}X$$

wherein A is H, HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC or R''''; B is H, SH or —NHR''', —N(R''')-(peptide) or R''''; X is SH or —NHR''', —N(R''')-(peptide) or R''''; Z is H or R''''; R', R'', R''' and R'''' are independently H or straight or branched chain or cyclic lower alkyl; n is 0, 1 or 2; and: (1) where B is —NHR''' or —N(R''')-(peptide), X is SH and n is 1 or 2; (2) where X is —NHR''' or —N(R''')-(peptide), B is SH and n is 1 or 2; (3) where B is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, X is SH and n is 0 or 1; (4) where A is H or R'''', then where B is SH, X is —NHR''' or —N(R''')-(peptide) and where X is SH, B is —NHR''' or —N(R''')-(peptide); (5) where X is H or R'''', A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH; (6) where Z is methyl, X is methyl, A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH and n is 0; and (7) where Z is SH and X is SH, n is not 0; and wherein the thiol moiety is in the reduced form.

In another embodiment, the invention provides peptide reagents capable of being radiolabeled with a radioisotope, for radiotherapy or for imaging sites within a mammalian body, each comprising a somatostatin anal. ogue that is covalently linked to a radiolabelbinding moiety of formula:

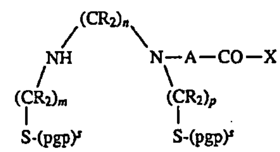


[for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolinic acid (Pic)-based moieties] or

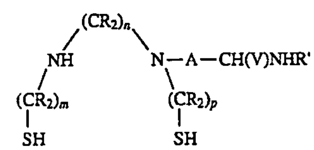


wherein X is H or a protecting group; (amino acid) is any amino acid and the radiolabel-binding moiety is covalently linked to the peptide. For purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolylamine (Pica)-based moieties. In a preferred embodiment, the amino acid is glycine and X is an acetamidomethyl protecting group.

Yet another embodiment of the invention provides peptide reagents capable of being radiolabeled with a radioisotope, for imaging sites within a mammalian body or for use as a radiotherapeutic agent, each comprising a somatostatin analogue that is covalently linked to a radiolabel-binding moiety that is a bisamino bisthiol radiolabel-binding moiety. The bisamino bisthiol radiolabel-binding moiety in this embodiment of the invention has the formula:



wherein each R can be independently H, CH_3 or C_2H_5; each $(pgp)^s$ can be independently a thiol protecting group or H; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; and X is peptide; or



wherein each R is independently H, CH_3 or C_2H_5; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; V is H or CO-peptide; R' is H or peptide; provided that when V is H, R' is peptide and when R' is H, V is CO-peptide. For purposes of this invention, radiolabel-binding moieties having these structures will be referred to as "BAT" moieties.

This invention provides methods for preparing peptide reagents of the invention by chemical synthesis in vitro. In a preferred embodiment, peptides are synthesized by solid phase peptide synthesis.

This invention provides reagents for preparing a radiolabled somatostatin receptor-binding agent comprising the somatostatin receptor-binding peptides of the invention covalently linked to a radiolabel-binding moiety. In a preferred embodiment, the reagent is radioactively labeled with Tc-99m. In another preferred embodiment, the reagent is radioactively labeled with ^{186}Re or ^{188}Re.

The invention also provides complexes of the linear peptide reagents of the invention with a radioisotope, as well as methods for radiolabeling the peptide reagents of the invention. For example, in one embodiment scintigraphic imaging agents provided by the invention comprise Tc-99m labeled complexes formed by reacting the peptide reagents of the invention with Tc-99m in the presence of a reducing agent. Preferred reducing agents include but are not limited

to dithionite ion, stannous ion and ferrous ion. Such Tc-99m complexes of the invention are also formed by labeling the peptide reagents of the invention with Tc-99m by ligand exchange of a prereduced Tc-99m complex as provided herein.

The invention also provides kits for preparing radiolabeled linear somatostain analogue peptides from the peptide reagents of the invention. Kits for radiolabeling the peptide reagents of the invention are comprised of a sealed vial containing a predetermined quantity of a peptide reagent of the invention and a sufficient amount of reducing agent to radiolabel the peptide. In a preferred embodiment, the radiolabeled somatostain analogue is a scintigraphic imaging agent. Also preferred is radiolabeling the peptide reagents of the invention with Tc-99m. Kits for preparing radiotheapeutic agents are also provided, wherein the preferred radioisotopes are rhenium-186 and rhenium-188.

This invention provides methods for using the radiolabeled peptide reagents of the invention diagniostically and therapeutically. In one embodiment of the invention, methods are provided for using scintigraphic imaging agents that are Tc-99m labeled peptide reagents for imaging sites within a mammalian body by obtaining in vivo gamma scintigraphic images. These methods comprise administering an effective diagnostic amount of Tc-99m labeled peptide reagents of the invention and detecting the gamma radiation emitted by the Tc-99m label localized at the site within the mammalian body.

The invention also provides methods for alleviating somatostatin-related diseases in animals, preferably humans, comprising administering a therapeutically effective amount of the radiolabeled somatostatin-binding peptide reagents of the invention to the animal. In preferred embodiments, the reagent is radioactively labeled with ^{186}Re or ^{188}Re.

The peptides and peptide reagents of the invention may also be comprised of a polyvalent linking moiety. Polyvalent linking moieties of the invention are comprised of at least 2 identical linker functional groups capable of covalently bonding to somatostatin analogue peptides or Tc-99m binding moieties. Preferred linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups. In preferred embodiments, the polyvalent linking moieties are comprised of bis-succinimidylmethylether (BSME), 4-(2,2-dimethylacetyl)benzoic acid (DMBA), N-[2-(N',N'-bis (2-succinimido-ethyl)aminoethyl)]-N^6,N^9-bis(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide (BAT—BS), tris(succinimidylethyl)amine (TSEA), bis-succinimidohexane (BSH), 4-(O-CH_2CO-Gly-Gly-Cys.amide)-2-methylpropiophenone (ETAC) or a derivative thereof.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides linear peptide reagents for preparing radiolabeled agents for radiodiagnostic and radiotherapeutic uses. The present invention provides linear peptides that are somatostatin analogues and that are not not constrained within a cyclic structure. Such somatostatin analogues thereby possess increased in vivo stability compared with native somatostatin. These linear peptides are themselves therapeutic agents for alleviating diseases and other ailments in animals including humans.

Also provided by the invention are linear peptides that may be radioiodinated or radioastatinated and which are thereby useful in radiotherapeutic and radiodiagnostic applications.

Another embodiment of these linear peptides that is provided by this invention are linear peptide reagents wherein the linear peptides of the invention are covalently linked to a radiolabel-binding moiety. Such linear peptide reagents are capable of being radiolabeled to provide radiodiagnostic or radiotherapeutic agents. One example of a radiodiagnostic application using the radiolabeled agents of the invention is scintigraphic imaging, wherein the location and extent of somatostatin receptor-bearing tumors may be determined.

The linear peptide reagents of the invention can also advantageously be radiolabeled with cytotoxic radioisotopes such as rhenium-186 or rhenium-188 for radiotherapeutic uses. The linear peptide reagents of the invention are also useful in preparing complexes with nonradioactive metals, said complexes being useful therapeutically.

The invention provides a method for using the somatostatin analogues of the invention to alleviate diseases or other ailments in animals, preferably humans. These diseases and ailments include but are not limited to diabetes and diabetes-related retinopathy, cirrhosis of the liver and hepatitis infection, bleeding ulcers and other gastrointestinal bleeding, pancreatitis, central nervous system disorders, endocrine disorders, Alzheimer's disease, acromegaly and other diseases and disorders related to the production of inappropriate levels of growth hormone in vivo, and cancer, particularly those cancers whose growth is dependent or influenced by growth hormone production. Dosages of the somatostatin analogues provided by the invention may be the same as those dosages of native somatostatin routinely used for treatment of the above or other diseases, or less of the compounds of the invention may be administered due to their longer in vivo half-life.

In embodiments of the invention useful as scintigraphic imaging agents, labeling with Tc-99m is an advantage of the present invention because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator. Other radionuclides may also be used in the practice of the invention as disclosed herein.

Radiotherapeutic embodiments of the invention, on the other hand, are advantageously labeled with cytotoxic radioisotopes including but not limited to scandium-47,copper-67, gallium-72, yttrium-90, iodine-125, iodine-131, samarium-153, gadolinium-159, dysprosium165, holmium-166, ytterbium-175, lutetium-177, rhenium-186, rhenium-188, astatine-211 and bismuth-212, most preferably ^{186}Re or ^{188}Re. Such embodiments are useful in the treatment of somatostatin-related diseases or other ailments in animals, preferably humans, including but not limited to cancer and other diseases characterized by the growth of malignant or benign tumors capable of binding somatostatin or somatostatin analogues via the expression of somatostatin receptors on the cell surface of cells comprising such tumors.

In the radiolabel-binding moieties and linear peptides covalently linked to such moieties that contain a thiol covalently linked to a thiol protecting groups [$(pgp)^S$] provided by the invention, the thiol-protecting groups may be the same or different and may be but are not limited to:

—CH_2—aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);

—CH—$(aryl)_2$,(aryl is phenyl or alkyl or alkyloxy substituted phenyl);

—C—$(aryl)_3$,(aryl is phenyl or alkyl or alkyloxy substituted phenyl);

—CH_2—(4-methoxyphenyl);

—$C(CH_3)_3$

—CH—(4-pyridyl)$(phenyl)_2$;

9-phenylfluorenyl;

—CH_2NHCOR (R is unsubstituted or substituted alkyl or aryl);

—CH_2—NHCOOR (R is unsubstituted or substituted alkyl or aryl);

—CONHR (R is unsubstituted or substituted alkyl or aryl);

CH_2—S—CH_2-phenyl

Preferred protecting groups have the formula —CH_2—NHCOR wherein R is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxycarbonyl. The most preferred protecting group is an acetamidomethyl group.

Each somatostatin receptor-binding linear peptide-containing embodiment of the invention is comprised of a sequence of amino acids. The term amino acid as used in this invention is intended to include all L- and D- amino acids, naturally occurring and otherwise. Reagents comprising somatostatin receptor-binding peptides provided by the invention include but are not limited to the following illustrative examples of the peptide embodiments of the invention:

$C_{Acm}GC_{Acm}GGGF_D$.Cpa.YW_DKTFT.amide
[DTPA].F_D.Cpa.YW_DKTFT(ε-K)GC.amide
maGGGF_D.Cpa.YW_DKTFT.amide
Ac.$C_{Acm}GC_{Acm}F_D$.Cpa.YW_DKTFT.amide
F_D.Cpa.YW_DKTFT$C_{Acm}GC_{Acm}$.amide
[DTPA].D-Nal.Cpa.YW_DKTFT(ε-K)GCKK.amide
AKCGGGF_D.Cpa.YW_DKTFT.amide
[DTPA]D-Nal.Cpa.YW_DKTFT(ε-K)GC.amide
F_D.Cpa.YW_DKTFT.GGG$C_{Acm}GC_{Acm}$.amide
[DTPA].Aca.F_D.Cpa.YW_DKTFT(ε-K)GC.amide
[DTPA].(ε-K)GCF_D.FYW_DKTFT.amide
Ac.CGCF_D.Cpa.YW_DKTFT.amide
F_D.Cpa.YW_DKTFTCGC.amide
[DTPA].(D-Nal.CYW_DKVCT$)_2$
Ac.F_D.FYW_DKTFT(ε-K)GC.amide
Ac.F_DFYW_DKTFTGGG(ε-K)GC.amide
F_D.Cpa.YW_DKTC.Nal.amide
K(BAT).D-Nal.$C_{Me}YW_D$KVC_{Me}T.amide
Ac.F_DFYW_DKTFGGG(ε-K)KC.amide
Pic.GC_{Acm}GGGF_D.Cpa.YW_DKTFT.amide
[DTPA].D-Nal.CYW_DKVCT.amide
(2-ketogulonyl)D-NalFYW_DKVCT.amide
F_D.Cpa.YW_DK.Abu.Nal.T(ε-K)GC.amide
[DTPA].K(BAT).D-Nal.$C_{Me}YW_D$KVC_{Me}T.amide
F_D.Cpa.YW_DKTFT(ε-K)GC.amide
[DTPA].F_DFYW_DKTFT(ε-K)GC.amide
AF_DCFW_DKTC_{Me}T(CH_2OH)
[DTPA].F_DGYW_DKTCT(CH_2OH)
[DTPA].Nal.SYW_DKVT.K(BAT).amide
[DTPA].Nal.SYW_DKVCT.amide

As used herein, the following amino acids and amino acid analogues are intended to be represented by the following abbreviations: Ac is an acetyl group; ma is mercaptoacetic acid group; Aca is 6-aminocaproic acid; Hey is homocysteine; Hhc is homohomocysteine, which is (3-mercaptopropyl)glycine; Pen is penicillamine; Mob is the sulfhydryl protecting group 4-methoxybenzyl; Acm is the sulfhydryl protecting group acetamidomethyl; Aib is aminoisobutyric acid; Nal is 2-naphthylalanine; Ain is 2-amino-indan-2-carboxylic acid; Hly is homolysine; Achxa is 4-amino-cyclohexylalanine; Amf is 4-aminomethylphenylalanine; Aec is S-(2-aminoethyl)cysteine; Ape is S-(3-aminopropyl)cysteine; Aes is 0-(2-aminoethyl)serine; Aps is O-(3-aminopropyl)serine; Abu is 2-aminobutyric acid; Nva is norvaline; Aca is 6-aminocaproic acid; F_D is D-phenylalanine; W_D is D-tryptophan; Y_D is D-tyrosine; Cpa is L-(4-chlorophenyl)alanine; Thp is 4-amino-tetrahydrothiopyran-4-carboxylic acid; D-Nal is D-2-naphthylalanine; Dpg is dipropylglycine; and Nle is norleucine. All naturally-occurring amino acids are abbreviated using standard abbreviations (which can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33. for the purposes of this invention, the naturally-occuring amino acids are characterized as lipophilic (alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan and valine, as well as S-alkylated derivatives of cysteine), hydrophilic (asparagine, glutamine, threonine, serine), acidic (glutamic acid and aspartic acid), basic (arginine, histidine and lysine). T(CH_2OH) represents a threoninol residue, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, incorporated into the peptide using the procedure of Neugebauer et al. (1990, *Peptides: Proceedings of the 11th American Peptide Symposium*, pp. 1020–21). ε-K is intended to represent a covalent linkage via the ε-amino group on the sidechain of a lysine residue. Pic is picolinoyl (pyridine-2-carbonyl); Pica is picolylamine (2-(aminomethyl)pyridine); [BAT] represents N^6,N^9-bis(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; K(BAT) and Lys(BAT) represent the amino acid lysine, acylated at the ε-amino group on the amino acid sidechain to [BAT]; [BAM] is (N^1,N^4-bis(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; [BAT-BM] is N-[2-(N',N'-bis(2-maleimidoethyl) aminoethyl]-N^9-(t-butoxycarbonyl)-N^6,N^9-bis(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; [BAT-BS]is N-[2-(N',N'-bis(2-succinimidethy)aminethy]-N^6, N^9-bis(2-mercapto 2-methypropyl)-6,9-diazannanamide; [BMME] is bis-maleimidomethylether; [BSME] is bis-succinimidomethylether; and [DTPA] is diethylenetriaminepentaacetic acid.

For the purposes of this invention the term "poly(N-carboxyalkyl)amine" in intended to describe a series of compounds exemplified by nitrilotriacetic acid, iminodiacetic acid, ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA).

For the purposes of this invention the term "polyoxyanion" is intended to encompass sulfates, phosphates, sulfonates, phosphonates and like compounds.

Linear somatostatin analogue peptides of the present invention can be chemically synthesized in vitro. Peptides of the present invention can generally advantageously be prepared on a peptide synthesizer. The peptides of this invention can be synthesized wherein the radiolabel-binding moiety is covalently linked to the peptide during chemical synthesis in vitro, using techniques well known to those with skill in the art. Such peptides covalently-linked to the radiolabel-binding moiety during synthesis are advantageous because specific sites of covalent linkage can be determined.

Radiolabel binding moieties of the invention may be introduced into the target linear somatostatin analogue peptides during peptide synthesis. For embodiments comprising picolinic acid [(Pic-); e.g., Pic-Gly-Cys(protecting group)-], the radiolabel-binding moiety can be synthesized as the last (i.e., amino-terminal) residue in the synthesis. In addition, the picolinic acid-containing radiolabel-binding moiety may

be covalently linked to the ϵ-amino group of lysine to give, for example, αN(Fmoc)-Lys-ϵN[Pic-Gly-Cys(protecting group)], which may be incorporated at any appropriate position in the peptide chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target somatostatin analogue peptide.

Similarly, the picolylamine (Pica)-containing radiolabel-binding moiety [-Cys(protecting group)-Gly-Pica] can be prepared during peptide synthesis by including the sequence [-Cys(protecting group)-Gly-] at the carboxyl terminus of the peptide chain. Following cleavage of the peptide from the resin the carboxyl terminus of the peptide is activated and coupled to picolylamine. This synthetic route requires that reactive side-chain functionalities remain masked (protected) and do not react during the conjugation of the picolylamine.

This invention also provides small linear synthetic peptides that are somatostatin analogues and incorporate bisamine bisthiol (BAT) chelators that may be labeled with Tc-99m.

This invention provides for the incorporation of these chelators into virtually any position in the peptide, via covalently linkage to any appropriate functional group of the peptide, except that the chelating moieties of the invention are not covalently linked to functional groups comprising the amino acid side chains of the amino acids B^1, B^2, B^3 or B^4.

In forming a complex of radioactive technetium with the reagents of this invention, the technetium complex, preferably a salt of Tc-99m pertechnetate, is reacted with the reagent in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. Means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a reagent of the invention to be labeled and a sufficient amount of reducing agent to label the reagent with Tc-99m. Alternatively, the complex may be formed by reacting a reagent of this invention with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex may be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the Tc-99m pertechnetate salts useful with the present invention are included the alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

In a preferred embodiment of the invention, a kit for preparing technetium-labeled peptides is provided. An appropriate amount of the peptide reagent is introduced into a vial containing a reducing agent, such as stannous chloride, in an amount sufficient to label the peptide with Tc-99m. An appropriate amount of a transfer ligand as described (such as tartrate, citrate, gluconate or mannitol, for example) can also be included. The kit may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The components of the kit may be in liquid, frozen or dry form. In a preferred embodiment, kit components are provided in lyophilized form.

Technetium-99m labeled imaging reagents according to the present invention may be prepared by the addition of an appropriate amount of Tc-99m or Tc-99m complex into the vials and reaction under conditions described in Example 2 hereinbelow.

Radioactively-labeled scintigraphic imaging agents provided by the present invention are provided having a suitable amount of radioactivity. In forming Tc-99m radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 millicurie (mCi) to 100 mCi per mL.

The imaging reagents provided by the present invention can be used for visualizing organs such as the kidney for diagnosing disorders in these organs, and tumors, in particular gastrointestinal tumors, myelomas, small cell lung carcinoma and other APUDomas, endocrine tumors such as medullary thyroid carcinomas and pituitary tumors, brain tumors such as meningiomas and astrocytomas, and tumors of the prostate, breast, colon, and ovaries can also be imaged. In accordance with this invention, the Tc-99m labeled peptide reagents are administered in a single unit injectable dose. The Tc-99m labeled peptide reagents provided by the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging in vivo can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

The somatostatin receptor-binding linear peptides and non-radioactive metal complexes of the linear peptide reagents of the invention may be used clinically to promote regression of certain types of tumors, particularly those that express somatostatin receptors. The linear somatostatin analogue peptides of the invention can also be used to reduce the hormonal hypersecretion that often accompanies certain cancers, such as the APUDomas. Peptides of the invention used as therapeutic agents may be administered by any appropriate route, including intravenous, intramuscular or by mouth, and in any acceptable pharmaceutical carrier, in doses ranging from about 0.1 to about 49 mg/kgbody weight/day.

This invention also provides peptides radiolabled with a cytotoxic radioisotope such as rhenium-186 or rhenium-188 that may be used for radiotherapy of certain tumors as described above. For this purpose, an amount of radioactive isotope from about 10 mCi to about 200 mCi may be administered via any suitable clinical route, preferably by intravenous injection.

The methods for making and labeling these compounds are more fully illustrated in the following Examples. These Examples illustrate certain aspects of the above-described method and advantageous results, and are shown by way of illustration and not limitation.

EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluore-

nylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/hydroxybenzotriazole (HBTU/HOBT), and using p-hydroxymethylphenoxymethylpolystyrene (HMP) resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Where appropriate, the following amino acid derivatives were synthesized. Homocysteine was prepared by alkaline hydrolysis of L-homocysteine lactone. Threoninol residues, wherein the carboxyl group of the amino acid is reduced to a primary alcohol, can be introduced into the peptides of the invention where appropriate using the procedure of Neugebauer et al. (1990, *Peptides: Proceedings of the 11th American Peptide Symposium*, pp. 1020–21). Fmoc. Hcy(Trt) and Fmoc.Pen(Trt) were prepared from the appropriate amino acids by tritylation with triphenylmethanol in TFA, followed by Fmoc derivitization as described by Atherton et al. (1989, *Solid Phase Peptide Synthesis*, IRL Press: Oxford). Fmoc.homohomocysteine(Trt) was prepared by reducing N,N-bis-Boc-glutamic acid-α-methyl ester with borane-THF, followed by mesylation and reaction with trityl-mercaptide, followed by removal of the Boc groups with BF_3OEt in acetic acid, and then Fmoc derivitization as described above. $PhCH_2CHBrCOOH$ was prepared by treating phenylalanine (in a solution of water and TFA/saturated with NaBr) with sodium nitrite, followed by distillation to recover the pure product.

Where appropriate, 2-chloroacetyl, 2-bromoacetyl and 2-bromo-3-phenylpropionyl groups were introduced either by using the appropriate 2-halo acid as the last residue coupled during SPPS, or by treating the N-terminus free amino acid peptide bound to the resin with either 2-halo acid/diisopropylcarbodiimide/N-hydroxysuccinimide/NMP or 2-halo acid anhydride/diisopropylethylamine/NMP.

Where appropriate, HPLC-purified 2-haloacylated peptides were cyclized by stirring an 0.1–1.0 mg/mL solution in phosphate or bicarbonate buffer or dilute ammonium hydroxide (pH 8.0), optionally containing 0.5–1.0 mM EDTA, or acetonitrile or THF for 1–48 h followed optionally by acidification with acetic acid, lyophilization and HPLC purification.

Where appropriate, [BAM](N^1,N^4-bis(2-mercapto-2-methylpropyl)-1,4,10-triazadecane) was conjugated to the peptide by first activating the peptide carboxylate with a mixture of diisopropylcarbodiimide/N-hydroxysuccinimide or HBTU/HOBt in DMF, NMP or methylene chloride, followed by coupling in the presence of diisopropylethylamine. After coupling, the conjugates were deprotected as described above.

Where appropriate, BSME adducts were prepared by reacting single thiol-containing peptides (5 to 50 mg/mL in DMF buffered to pH 7 with N-methylmorpholine or N-ethyl-morpholine, or 50 mM sodium phosphate buffer, pH 7–8, optionally containing 0.5 mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BMME (bis-maleimidomethylether) pre-dissolved in acetonitrile at room temperature for approximately 1–18 hours. The solution was concentrated and the product was purified by HPLC.

Where appropriate, TSEA adducts were prepared by reacting single thiol-containing peptide (at concentrations of 10 to 100 mg/mL peptide in DMF buffered to pH 7 with N-methylmorpholine or N-ethylmorpholine, or 5 to 50 mg/mL peptide in 50 mM sodium phosphate, pH 7–8, optionally containing 0.5mM EDTA or DMF or THF or acetonitrile) with 0.33 molar equivalents of TMEA (tris(2-maleimidoethyl)amine) pre-dissolved in acetonitrile or DMF, with or without 1 molar equivalent of triethanolamine, at room temperature for approximately 1–18h. Such reaction mixtures containing adducts were concentrated and the adducts were then purified using HPLC.

Where appropriate, BAT-BS (N-[2-(N',N'-bis(2-succinimidoethyl) aminoethyl)]-N^6,N^9-bis (2-methyl-2-mercaptopropyl)-6,9-diazanonanamide) adducts were prepared by reacting single thiol-containing peptide (at concentrations of 2 to 50 mg/mL peptide in DMF buffered to pH 7 with N-methyl-morpholine or N-ethyl-morpholine, or in 50 mM sodium phosphate (pH 7–8), optionally containing 0.5 mM EDTA or DMF or THF or acetonitrile) with 0.5 molar equivalents of BAT-BM (N-[2-(N',N'-bis(2-maleimidoethyl)aminoethyl)]-N^9-(t-butoxycarbonyl)-N^6,N^9-bis(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide) pre-dissolved in acetonitrile or THF, at room temperature for approximately 1–18 h. The solution was then evaporated to dryness and [BAT-BS]-peptide conjugates deprotected by treatment with 10mL TFA and 0.2 mL triethylsilane for 1 h. The solution was concentrated, the product adducts precipitated with ether, and then purified by HPLC.

Where appropriate, the [DTPA]moiety can be introduced using the method of Bakker et al. (1991,Life Sci. 49: 1583–1591,hereby incorporated by reference).

Resin-bound products were routinely cleaved using a solution of trifluoroacetic acid or trifluoroacetic acid and methylene chloride, optionally containing water, thioanisole, ethanedithiol, and triethylsilane, prepared in ratios of 100:5:5:2.5:2 for 0.5–3 h at room temperature. Crude peptides were purified by preparative high pressure liquid chromatography (HPLC) using a Waters Delta Pak C18 column and gradient elution using 0.1% trifluoroacetic acid (TFA) in water modified with acetonitrile. Acetonitrile was evaporated from the eluted fractions which were then lyophilized. The identity of each product was confirmed by fast atom bombardment mass spectroscopy (FABMS) or by electrospray mass spectroscopy (ESMS).

Somatostatin analogues synthesized as provided herein, as well as the products of such synthesis identified by FABMS, are shown in Table I below.

EXAMPLE 2

A General Method for Radiolabeling with Tc-99m

0.1 mg of a peptide prepared as in Example 2 was dissolved in 0.1 mL of water or 50/50 ethanol/water or phosphate-buffered saline or 50 mM potassium phosphate buffer (pH =5, 6 or 7.4). Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc.) with 1.0 mL of Tc-99m sodium pertechnetate containing up to 200 mCi and allowed to stand for 15 minutes at room temperature. 25 μl of Tc-99m gluceptate was then added to the peptide and the reaction allowed to proceed at room temperature or at 100° C. for 15–30 min and then filtered through a 0.2 μm filter.

The Tc-99m labeled peptide purity was determined by HPLC using the following conditions: a Waters Delta Pak RP-18, 5μ, 4.6 mm×220 mm analytical column was loaded with each radiolabeled peptide, and the peptides eluted at a solvent flow rate equal to 1 mL/min. Gradient elution was performed beginning with 100% solvent A (0.1% CF_3COOH/H_2O) and ending with 100% solvent B_{90} (0.1% $CF_3COOH/90\%$ CH_3CN/H_2O) over the course of 10–20 min.

Radioactive components were detected using an in-line radiometric detector linked to an integrating recorder. Tc-99 m gluceptate and Tc-99 m sodium pertechnetate elute between 1 and 4 minutes under these conditions, whereas the Tc-99m labeled peptides eluted after a much greater amount of time, as illustrated in Table I below.

TABLE I

Peptide	MH$^+$	RCY (%)	R_t (min)
C$_{Acm}$GC$_{Acm}$GGGF$_D$.Cpa.YW$_D$KTFT.amide	1749	97[4]	15.7[2]
[DPTA].F$_D$.Cpa.YW$_D$KTFT(ε-K)GC.amide	1837	97[5]	15.5[2]
ma.GGGF$_D$.Cpa.YW$_D$KTFT.amide	1417	98[4]	12.2[3]
Ac.C$_{Acm}$GC$_{Acm}$F$_D$.Cpa.YW$_D$KTFT.amide	1619	75[4]	17.1, 17.5[2]
F$_D$.Cpa.YW$_D$KTFTC$_{Acm}$GC$_{Acm}$.amide	1577	93[3]	12.1[3]
[DPTA].D-Nal.Cpa.YW$_D$KTFT(ε-K)GCKK.amide	2143	nd	nd
AKCGGGF$_D$.Cpa.YW$_D$KTFT.amide	1612	98[5]	15–16[2]
[DPTA].D-Nal.Cpa.YW$_D$KTFT(ε-K)GC.amide	1887	97[5]	16.2[2]
F$_D$.Cpa.YW$_D$KTFT.GGGC$_{Acm}$GC$_{Acm}$.amide	1749	76[3]	17.7, 18.0[1]
[DTPA].Aca.F$_D$.Cpa.YW$_D$KTFT(ε-K)GC.amide	1950	97[2]	11.5[3]
[DTPA].(ε-K)GCF$_D$.FYW$_D$KTFT.amide	1802	97[2]	11.5[3]
Ac.CGCF$_D$.Cpa.YW$_D$KTFT.amide	1477	98[6]	18.1[2]
F$_D$.Cpa.YW$_D$KTFTCGC.amide	1435	99[6]	16.8, 17.0[2]
[DTPA].(D-Nal.CYW$_D$KVCT)$_2$	2554	97[6]	11.8–12.4[3]
Ac.F$_D$.FYW$_D$KTFT(ε-K)GC.amide	1469	96[2]	12.1, 12.6[3]
Ac.F$_D$FYW$_D$KTFTGGG(ε-K)GC.amide	1640	98[2]	11.9, 12.4[3]
F$_D$.Cpa.YW$_D$KTC.Nal.amide	1224	88[6]	18.6, 20.4[2]
K.[BAT].D-Nal.C$_{Me}$YW$_D$KVC$_{Me}$T.amide	1573	97[6]	12.5[3]
Ac.F$_D$FYW$_D$KTFGGG(ε-K)KC.amide	1710	98[6]	15.9[2]
Pic.GC$_{Acm}$GGGF$_D$.Cpa.YW$_D$KTFT.amide	1681	98[6]	13.8–16.8[1]
[DTPA].D-Nal.CYW$_D$KVCT.amide	1473	97[6]	11.0[3]
(2-ketogulonyl)-D-NalFYW$_D$KVCT.amide	1318	98[2]	12.4, 13.0[3]
F$_D$.Cpa.YW$_D$K.Abu.Nal.T(ε-K)GC.amide	1495	95[5]	16.5[2]
[DTPA].K.[BAT].D-Nal.C$_{Me}$YW$_D$KVC$_{Me}$T.amide	1949	96[6]	12.3[3]
F$_D$.Cpa.YW$_D$KTFT(ε-K)GC.amide	1461	98[5]	15.8[2]
[DTPA].F$_D$FYW$_D$KTFT(ε-K)GC.amide	1801	97[3]	11.3[3]
AF$_D$CFW$_D$KTC$_{Me}$T(CH$_2$OH)	1106	99[1]	11.3–11.9[3]
[DTPA].F$_D$GYW$_D$KTCT(CH$_2$OH)	nd	96[2]	10.6[1]
[DTPA].Nal.SYW$_D$KVTK.[BAT].amide	1801	96[2]	12.0[3]
[DPTA].Nal.SYW$_D$KVCT.amide	1457	95[6]	11.6[3]

*The following labeling conditions were used with the appropriate peptides:
1. The peptide is dissolved in 50 mM potassium phosphate buffer (pH 7.4) and labeled at room temperature.
2. The peptide is dissolved in water and labeled at room temperature.
3. The peptide is dissolved in water and labeled at 100° C.
4. The peptide is dissolved in 50% ethanol/water and labeled at 100° C.
5. The peptide is dissolved in 10% hydroxypropylcyclodextrin and labeled at room temperature.
6. The peptide is dissolved in 50% ethanol/water labeled at room temperature.

**HPLC methods:
general:
solvent A = 0.1% CF3COOH/H$_2$O
solvent B$_{90}$ = 0.1% CF$_3$COOH/90% CH$_3$CN/H$_2$O
solvent flow rate = 1 mL/min
Vydak column = Vydak 218TP54 RP-18, 5μ × 220 mm × 4.6 mm analytical column with guard column
Waters column = Waters Delta-Pak C18, 5 μm, 39 × 150 mm
Method 1: Vydak column 100% A to 100% B$_{90}$ in 10 min
Method 2: Waters column 100% A to 100% B$_{90}$ in 20 min
Method 3: Waters column 100% A to 100% B$_{90}$ in 10 min

Single-letter abbreviations for amino acids can be found in G. Zubay, Biochemistry (2d. ed.), 1988 (MacMillen Publishing: New York) p. 33; Ac = acetyl; Acm = acetamidomethyl; ma = mercaptoacetic acid; Aca = 6-aminocaproic acid; Hly = homolysine; Apc = L-[S—(3-aminopropyl)cysteine; F$_D$ = D-phenylalanine; W$_D$ = D-tryptophan; Y$_D$ = D-tyrosine; Cpa = L—(4-chlorophenyl)alanine; D-Nal =D-2-naphthylalanine; Nle = norleucine; Hcy = homocysteine; Hhc = homohomocysteine; Pen = penicillamine; Aib = aminoisobutyric acid; Nal = 2-naphthylalanine; D-Nal = D-2-naphthylalanine; Ain = 2-aminoindane-2-carboxylic acid; Achxa = 4-amino-cyclohexylalanine; Amf = 4-aminomethyl-phenylalanine; Aec = S—(2-aminoethyl)cysteine; Apc = S—(3-aminopropyl)cysteine; Aes = O—(2-aminoethyl)serine; Aps = O—(3-aminopropyl)serine; Abu = 2-aminobutyric acid; Nva = norvaline; T(CH$_2$OH) = threoninol (on which the carboxylic acid moiety has been reduced to a primary alcohol); ε-K = a lysine residue in a peptide in which the peptide bond involves the ε-amino group on the lysine sidechain rather than the α-amino group; Pic = picolinoyl (pyridine-2-carbonyl); Pica = picolylamine (2-(aminomethyl)pyridine); BAT =N^6,N^9-bis(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; BAT (protected) = N^9-(t-butoxycarbonyl)-N^6,N^9-bis(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanoicacid; BAM = N^1,N^4-bis(2-mercapto-2-methylpropyl)-1,4,10-triazadecane; BAM (protected) = N^1-(t-butoxycarbonyl)-N^1,N^4-bis(2-methyl-2-triphenylmethylthiopropyl)-1,4,10-triazadecane; [BAT- BM] = N-[2-(N',N'-bis(2-maleimidoethyl)aminoethyl]-N^9-(t-butoxycarbonyl)-N^6,N^9-bis(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; [BAT-BS] = N-[2-(N',N'-bis(2-succinimidoethyl)aminoethyl]-N^6,N^9-bis(2-mercapto-2-methylpropyl)-6,9-diazanonanamide; [BMME] = bis-maleimidomethylether; [BSME] = bis-succinimidomethylether; [DTPA] =diethylenetriaminepentaacetic acid.
RCY (%) = radiochemical yield (determined by HPLC)

EXAMPLE 3

Inhibition of Binding of [^{125}I-Tyr^{11}]somatostatin-14 to AR42J Rat Pancreatic Tumor Cell Membranes

The ability of various somatostatin analogues of the invention to bind to somatostatin receptors in vitro was demonstrated by assaying the ability of such analogues to inhibit binding of a radiolabeled somatostatin analogue to somatostatin receptor-containing cell membranes. The rat pancreatic tumor cell line AR42J which expresses the somatostatin receptor was cultured in Dulbecco's minimal essential media (DMEM) supplemented with 10% fetal bovine serum (FBS) and 8 mM glutamine in a humdified 5% CO_2 atmosphere at 37° C. in T-flasks. Harvested cells were homogenized in cold 50 mM Tris-HCl buffer (pH 7.4) and the homogenate then centrifuged at 39,000 g for 10 min at 4° C. Pellets were washed once with buffer and then resuspended in an ice-cold solution of 10 mM Tris-HCl (pH 7.4). Equal aliquots of this cell membrane preparation were incubated with [^{125}I-Tyr^{11}]somatostatin-14 (at a final concentration of 0.5nM and 750,000 cpm/mL, at a specific activity of 2000 Ci/mmol, Amersham, Arlington Heights, Ill.) and peptide at a final concentration of from 10^{-11} M to 10^{-6} M in a solution of 50 mM HEPES (pH 7.4) containing 1% bovine serum albumin (BSA), 5 mM $MgCl_2$, Trasylol (200,000 International Units), bacitracin (0.02 mg/mL) and phenylmethylsulfonylfluoride (0.02 mg/mL) for 25 min at 30° C. Using a filtration manifold, this mixture was filtered through a polyethyleneimine-washed GC/F filter (Whatman, Maidstone, England), and the residue remaining on the filter wastled thrice with 5 mL cold HEPES buffer. The filter and a sample of the filter washings were then counted in a gamma counter. To assess non-specific binding, the assay was performed in the presence of unlabeled somatostatin-14 at 200 nM. Data analysis including Hill plots of the data provided inhibition constants (see Bylund & Yamamura, "Methods of receptor binding", in *Methods in Neurotransmitter Receptor Analysis*, Yamamura et al., eds., Raven Press: New York, 1990).

These results are presented in the following Table. The data show that the peptides of the instant invention have a high affinity of binding for somatostatin receptors.

TABLE II

	MH^+	K_i (nM)
Peptide		
$C_{Acm}GC_{Acm}GGGF_D$.Cpa.YW_DKTFT.amide		<0.01
[DTPA].F_D.CPa.YW_DKTFT(ϵ-K)GC.amide		0.24
maGGGF_D.Cpa.YW_DKTFT.amide		0.25
Ac.$C_{Acm}GC_{Acm}F_D$.CPa.YW_DKTFT.amide		0.73
F_D.CPa.YW_DKTFT$C_{Acm}GC_{Acm}$.amide		0.85
[DTPA].D-Nal.Cpa.YW_DKTFT(ϵ-K)GCKK.amide		1.3
AKCGGGF_D.Cpa.YW_DKTFT.amide		1.4
[DTPA].D-Nal.Cpa.YW_DKTFT(ϵ-K)GC.amide		2.0
F_D.Cpa.YW_DKTFT.GGG$C_{Acm}GC_{Acm}$.amide		2.4
[DTPA].Aca.F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide		2.6
[DTPA].(ϵ-K)GCF_D.FYW_DKTFT.amide		3.3
Ac.CGCF_D.Cpa.YW_DKTFT.amide		4.4
F_D.Cpa.YW_DKTFTCGC.amide		4.8
[DTPA].(D-Nal.CYW_DKVCT)$_2$		7.2
Ac.F_D.FYW_DKTFT(ϵ-K)GC.amide		7.9
Ac.F_DFYW_DKTFTGGG(ϵ-K)GC.amide		8.2
F_D.Cpa.YW_DKTC.Nal.amide		8.2
K.[BAT].D-Nal.$C_{Me}YW_D$KVC_{Me}T.amide		9.9
[Re═O]-complexed Peptides		
[DTPA].D-Nal.Cpa.YW_DKTFT(ϵ-K)GC.amide	2085	0.007
[DTPA].F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide	2036	0.027
F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide	nd	0.36
F_D.CPa.YW_DKTFTCGC.amide	1635	0.37
$C_{Acm}GC_{Acm}GGGF_D$.Cpa.YW_DKTFT.amide	1807	0.43
AKCGGGF_D.Cpa.YW_DKTFT.amide	1812	0.76
maGGGF_D.Cpa.YW_DKTFT.amide	1618	0.97
Ac.F_DFYW_DKTFT(ϵ-K)GC.amide	1688	1.5
AKCGGGF_DFYW_DKTFT.amide	1812	2.9
F_D.Cpa.YW_DK.Abu.Nal.T(ϵ-K)GC.amide	1695	3.3
Ac.CGCF_D.Cpa.YW_DKTFT.amide	1677	4.1
Ac.F_DFYW_DKTFGGG(ϵ-K)KC.amide	1911	6.1
Ac.F_DFYW_DKTFTGGG(ϵ-K)GC.amide	1840	8.1
[DTPA].Aca.F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide	2149	8.2

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A composition of matter that is linear somatostatin receptor-binding peptide reagent having the formula:

$$X^1\text{-}A^1A^2\text{-}B^1B^2B^3B^4\text{-}C^1C^2\text{-}X^2$$

wherein

X^1 and X^2 are each independently hydrophilic moieties wherein X^1 is an amino acid, or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide, or an oligosaccharide comprising 10 or fewer saccharide units, or a polyoxyanion, and X^2 is a polyoxyanion, or an amino acid, or a peptide having an amino acid sequence of no more than 10 residues (including peptides wherein the carboxyl group of the

carboxyl-terminal amino acid is reduced to an alcohol), or a monosaccharide or an oligosaccharide comprising 10 or fewer saccharide units;

A^1, A^2 and C^1 are each independently a lipophilic D- or L-amino acid, or S-alkylated cysteine, penicillamine, homocysteine or homohomocysteine;

B^1 is D- or L-Phe, or D- or L-Tyr, or D- or L-Nal, or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys, or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 and C^2 are each independently D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle,

Leu, Nva, Nal or Aib or substituted derivatives thereof; and wherein the somatostatin receptor-binding peptide reagent does not comprise a radiolabel chelating moiety.

2. The composition of matter of claim **1** wherein B^1 is phenylalanine or tyrosine, B^2 is D-tryptophan, B^3 is lysine and B^4 is threonine or valine.

3. The composition of matter of claim **1** further comprising a polyvalent linking moiety that is covalently linked to a multiplicity of the somatostatin receptor-binding peptides to form a multimeric polyvalent somatostatin receptor binding agent, wherein the molecular weight of the multimeric polyvalent somatostatin receptor binding agent is less than about 20,000 daltons.

4. The composition of matter of claim **3** wherein the polyvalent linking moiety is bis-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, N-[2-(N',N'-bis(2-succinimidoethyl)aminoethyl)]-N^6, N^9-bis(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, tris(succinimidylethyl)amine or a derivative thereof.

5. The composition of matter of claim **1** wherein the somatostatin receptor-binding peptide is chemically synthesized in vitro.

6. The composition of matter of claim **5** wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

7. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the somatostatin receptor binding peptide of claim **1** to the animal.

8. The method of claim **7** wherein the animal is a human.

9. A composition of matter that is a linear somatostatin receptorbinding peptide reagent having the formula:

$$X^1\text{-}A^1A^2\text{-}B^1B^2B^3B^4\text{-}C^1C^2\text{-}X^2$$

wherein

X^1 is H, lower alkyl or substituted alkyl, aryl or substituted aryl, alkanoyl or substituted alkanoyl, aroyl or substituted aroyl, or a hydrophilic moiety;

A^1, A^2 and C^1 are each independently a lipophilic D- or L-amino acid, or S-alkylated cysteine, penicillamine, homocysteine or homohomocysteine;

B^1 is D- or L-Phe, or D- or L-Tyr, or D- or L-Nal, or Ain or substituted derivatives thereof;

B^2 is D- or L-Trp or substituted derivatives thereof;

B^3 is D- or L-Lys, or Hly, Achxa, Amf, Aec, Apc, Aes, Aps or substituted derivatives thereof;

B^4 and C^2 are each independently D- or L-Thr, Ser, Val, Phe, Ile, Abu, Nle, Leu, Nva, Nal or Aib or substituted derivatives thereof;

X^2 is —$COOR^9$, —CH_2OH, CH_2COOR^9,or —$CON(R^9)_2$, where each R^9 is independently H, lower linear or cyclic alkyl or substituted derivatives thereof or substituted with a hydrophilic moiety;

and wherein the somatostatin receptor binding peptide is covalently linked to a radiolabel-binding moiety, wherein the radiolabel-binding moiety is not covalently linked to the moieties B^1, B^2, B^3 or B^4 of the peptide and wherein the radiolabel binding moiety is capable of binding Tc-99m, Re-186 or Re-188.

10. The composition of matter of claim **9** wherein X^1 is a an amino acid, or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide, or an oligosaccharide comprising 10 or fewer saccharide units, or a polyoxyanion and X^2 is a polyoxyanion, or an amino acid, or an amino acid, or a peptide having an amino acid sequence of no more than 10 residues, or a monosaccharide, or an oligosaccharide comprising 10 or fewer saccharide units.

11. The composition of matter of claim **9** wherein B^1 is phenylalanine or tyrosine, B^2 is D-otryptophan, B^3 is lysine and B^4 is threonine or valine.

12. The composition of matter of claim **9** wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of the somatostatin receptor binding peptides and also covalently linked to a multiplicity of radiolabelbinding moieties to comprise a reagent for preparing a multimeric polyvalent somatostatin receptor binding reagent, wherein the molecular weight of the multimeric polyvalent somatostatin receptor binding reagent is less than about 20,000 daltons.

13. The composition of matter of claim **12** wherein the polyvalent linking moiety is bis-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, N-[2-(N',N'-bis(2-succinimidethy)aminethy)]-N^6,N^9-bis(2-methyl-2-mercaptprpy)-6,9-diazanonanamide, tris(succinimidylethyl)amine or a derivative thereof.

14. A scintigraphic imaging agent comprising the composition of matter of claim **9** radiolabeled with technetium-99m.

15. A radiotherapeutic agent comprising the composition of matter of claim **9** radiolabeled with a cytotoxic radioisotope selected from the group consisting of rhenium-186 and rhenium-188.

16. A complex formed by reacting the composition of matter of claim **9** with technetium-99m in the presence of a reducing agent.

17. The complex of claim **16** ,wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

18. A complex formed by labeling the composition of matter of claim **9** with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

19. A composition comprising the composition of matter of claim **9** and a stannous ion.

20. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the composition of matter of claim **9** and a sufficient amount of reducing agent to label the reagent with technetium-99m.

21. A method for labeling a composition of matter according to claim **9** comprising reacting the composition of matter with technetium-99m in the presence of a reducing agent.

22. The method of claim **21**, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

23. A composition of matter according to claim **9** wherein the somatostatin receptor-binding peptide is chemically synthesized in vitro.

24. A composition of matter according to claim **23** wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

25. A composition of matter according to claim **23** wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during in vitro chemical synthesis.

26. A composition of matter according to claim 25 wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.

27. A reagent for preparing a scintigraphic imaging agent for imaging sites within a mammalian body comprising a composition of matter according to claim 9 that is a somatostatin receptor-binding peptide and a radiolabel-binding moiety covalently linked thereto, the radiolabel-binding moiety having the formula:

C(pgp)^s-(aa)-C(pgp)^s

wherein (pgp)^s is H or a thiol protecting group and (aa) is an amine acid; or

A-CZ(B)-[C(R'R")]$_n$-X

wherein

A is H, HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC or R"";

B is H, SH, -NHR'", -N(R'")-(peptide), or R"";

X is H, SH, -NHR'", -N(R'")-(peptide) or R"";

Z is H or R"";

R', R", R'" and R"" are independemly H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

and

where B is -NHR'" or -N(R'")-(peptide), X is SH, and n is 1 or 2;

where X is -NHR'" or -N(R'")-(peptide), B is SH, and n is 1 or 2;

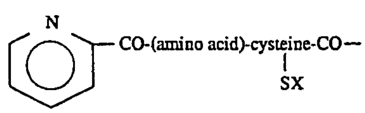
where B is H or R"", A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, X is SH, and n is 0 or 1;

where A is H or R"", then where B is SH, X is -NHR'" or-N(R'")-(peptide) and where X is SH, B is -NHR'"or-N(R'"-(peptide);

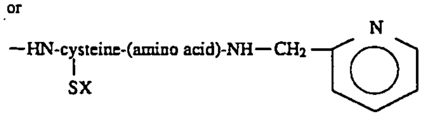
where X is H or R"", A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC and B is SH;

where Z is methyl, X is methyl, A is HOOC, H_2NOC, (peptide)-NHOC, (peptide)-OOC, B is SH and n is 0;

where B is SH and X is SH, n is not 0; or



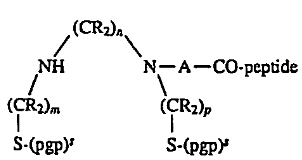
or



wherein

X=H or a protecting group;

(amino acid)=any amino acid; or



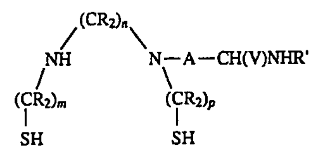
wherein

each R is independently H, CH_3 or C_2H_5;

each (pgp)^s is independently a thiol protecting group or H;

m, n and p are independently 2 or 3;

A=linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;



wherein

each R is independently H, CH_3 or C_2H_5;

m, n and p are independently 2 or 3;

A=linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof;

V=H or—CO-peptide;

R'=H or peptide;

and wherein when V=H, R'=peptide and when R'=H, V=—CO-peptide; wherein each R is independently H, lower alkyl having 1 to 6 carbon atoms, phenyl, or phenyl substituted with lower alkyl or lower alkoxy, and wherein each n is independently 1 or 2.

28. The reagent of claim 27 wherein the cysteine of the radiolabel-binding moiety having formula

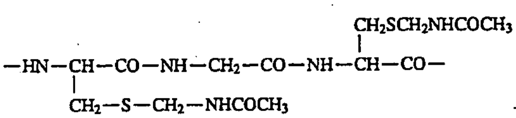
C(pgp)-(aa)-C(pgp)^s

has a protecting group of the formula

—CH_2-NH-CO-R

wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-,3-,4-pyridyl, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl.

29. The reagent of claim 32 wherein the radiolabel-binding moiety C(pgp)^s-(aa)-C(pgp)^s has the formula:



30. A scintigraphic imaging agent that is the reagent of claim 27 radiolabeled with technetium-99m.

31. A complex formed by reacting the reagent of claim 27 with technetium-99m in the presence of a reducing agent.

32. The complex of claim 31, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

33. A complex formed by labeling the reagent of claim 27 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

34. A composition comprising the reagent of claim 27 and a stannous ion.

35. A kit for preparing a radiopharmaceutical preparation, said kit comprising a sealed vial containing a predetermined quantity of the reagent of claim 27 and a sufficient amount of reducing agent to label the reagent with technetium-99m.

36. A method for labeling a reagent according to claim 27 comprising reacting the reagent with technetium-99m in the presence of a reducing agent.

37. The method of claim 36, wherein the reducing agent is selected from the group consisting of a dithionite ion, a stannous ion and a ferrous ion.

38. A method for imaging a site within a mammalian body comprising administering an effective diagnostic amount of

the reagent of claim 30 and detecting the technetium-99m localized at the site in the mammalian body.

39. The reagent according to claim **27** wherein the somatostatin receptor-binding peptide comprising the reagent is chemically synthesized in vitro.

40. The reagent according to claim **39** wherein the somatostatin receptor-binding peptide is synthesized by solid phase peptide synthesis.

41. The reagent according to claim **39** wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during in vitro chemical synthesis.

42. The reagent according to claim **41** wherein the radiolabel-binding moiety is covalently linked to the somatostatin receptor-binding peptide during solid phase peptide synthesis.

43. The reagent of claim **27** wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of the somatostatin receptor binding peptides and also covalently linked to a multiplicity of radiolabel-binding moieties to comprise a reagent for preparing a multimeric polyvalent somatostatin receptor binding reagent, wherein the molecular weight of the multimeric polyvalent somatostatin receptor binding reagent is less than about 20,000 daltons.

44. The reagent of claim **43** wherein the polyvalent linking moiety is bis-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, N-[2-(N',N'-bis(2-succinimidethyl)aminethyl)]-N^6,N^9-bis(2-methyl-2-mercaptopropyl)-6,9-diazannanamide, tris(succinimidylethyl)amine or a derivative thereof.

45. A composition of matter according to claim **9** wherein the radiolabel binding moiety forms a neutral complex with technetium-99m.

46. A composition of matter according to claim **9** radiolabeled with technetium-99m.

47. A composition of matter according to claim **9** radiolabeled with a radioisotope selected from the group consisting of, rhenium-186 and rhenium-188.

48. A composition of matter comprising a somatostatin receptor-binding peptide reagent selected from the group consisting of reagents having the formula:

$C_{Acm}GC_{Acm}GGGF_D$.Cpa.YW_DKTFT.amide
[DTPA].F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide
ma. $GGGF_D$.Cpa.YW_DKTFT.amide
Ac.$C_{Acm}GC_{Acm}F_D$.Cpa.YW_DKTFT.amide
F_D.Cpa.YW_DKTFT$C_{Acm}GC_{Acm}$.amide
[DTPA].D-Nal.Cpa.YW_DKTFT(ϵ-K)GC.amide
AKCGGGF_D.Cpa.YW_DKTFT.amide [DTPA].D-Nal-.Cpa.YW_DKTFT(ϵ-K)GC.amide
F_D.Cpa.YW_DKTFT.GGG$C_{Acm}GC_{Acm}$.amide
[DTPA].Aca.F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide
[DTPA].(ϵ-K)GCF_D.FYW_DKTFT.amide
Ac.CGCF_D.Cpa.YW_DKTFT.amide
F_D.Cpa.YW_DKTFTCGC.amide
[DTPA].(D-Nal.CYW_DKVCT)$_2$
Ac.F_D.FYW_DKTFT(ϵ-K)GC.amide
Ac.F_DFYW_DKTFTGGG(ϵ-K)GC.amide
$C_{Acm}GC_{Acm}GGGF_D$.Cpa.YW_DKTFT.amide
F_D.Cpa.YW_DKTC.Nal.amide
K.[BAT].D-Nal. $C_{Me}YW_D$KVC_{Me}T.amide
Ac.F_DFYW_DKTFGGG(ϵ-K)KC.amide
Pic.GC_{Acm}GGGF_D.Cpa.YW_DKTFT.amide [DTPA].D-Nal-.CYW_DKVCT.amide
(2-ketogulonyl)-D-NalFYW_DKVCT.amide
F_D.Cpa.YW_DK.Abu.Nal.T(ϵ-K)GC.amide
[DTPA].K.[BAT].D-Nal.$C_{Me}YW_D$KVC_{Me}T.amide
F_D.Cpa.YW_DKTFT(ϵ-K)GC.amide
[DTPA].F_DFYW_DKTFT(ϵ-K)GC.amide
AF_DCFW_DKTC_{Me}T(CH_2OH)
[DTPA].F_DGYW_DKTCT (CH_2OH)
[DTPA].Nal.SYW_DKVTK.[BAT]. amide
[DTPA].Nal.SYW_DKVCT.amide.

49. The composition of matter of claim **48** radiolabeled with technetium-99m.

50. The composition of matter of claim **48** radiolabeled with a radioisotope selected from the group consisting of rhenium-186 and rhenium-188.

51. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the composition of matter of claim **1** to the animal.

52. The method of claim **51** wherein the animal is a human.

53. The method of claim **51** wherein the therapeutically effective amount of the composition of matter administered to the animal is from about 0.1 to about 49 mg/kg body weight/day.

54. A method for alleviating a somatostatin-related disease in an animal comprising administering a therapeutically effective amount of the radiotherapeutic agent of claim **15** to the animal.

55. The method of claim **54** wherein the animal is a human.

56. The method of claim **54** wherein the therapeutically effective amount of the radiotherapeutic agent administered to the animal is from about 10 to about 200 mCi.

57. A pharmaceutical composition comprising the radiotherapeutic agent of claim **15** in a pharmaceutically-acceptable carrier.

58. A pharmaceutical composition comprising a composition of matter according to claim **1** that is a somatostatin receptor binding peptide in a pharmaceutically acceptable carrier.

59. A complex formed by reacting the composition of matter of claim **9** with a non-radioactive metal.

60. The complex of claim **59** wherein the non-radioactive metal is rhenium.

61. A complex formed by reacting the composition of matter of claim **12** with a non-radioactive metal.

62. The complex of claim **59** wherein the non-radioactive metal is rhenium.

63. A complex formed by reacting the composition of matter of claim **27** with a non-radioactive metal.

64. The complex of claim **63** wherein the non-radioactive metal is rhenium.

65. A complex formed by reacting the composition of matter of claim **43** with a non-radioactive metal.

66. The complex of claim **38** wherein the non-radioactive metal is rhenium.

67. A complex formed by reacting the composition of matter of claim **48** with a non-radioactive metal.

68. The complex of claim **67** wherein the non-radioactive metal is rhenium.

* * * * *